WEST

Search Results - Record(s) 1 through 2 of 2 returned.

☐ 1. Document ID: AU 9950311 A, DE 19900503 A1, WO 200040263 A1

L2: Entry 1 of 2   File: DWPI   Jul 24, 2000

DERWENT-ACC-NO: 2000-466995
DERWENT-WEEK: 200052
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TITLE: Use of composition containing anti-Fas antibody, for treating
e.g. toxic epidermal necrolysis or hepatitis, inhibits interaction
between Fas receptor and ligand

INVENTOR: FRENCH, L E ; TSCHOPP, J ; VIARD, I ; FRENCH, E L

PRIORITY-DATA: 1999DE-1000503 (January 8, 1999)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 9950311 A	July 24, 2000	N/A	000	A61K039/395
DE 19900503 A1	July 13, 2000	N/A	019	A61K039/395
WO 200040263 A1	July 13, 2000	G	000	A61K039/395

INT-CL (IPC): A61K 39/395; C07K 16/06; C07K 19/00; G01N 33/68

| Full | Title | Citation | Front | Review | Classification | Date | Reference | Claims | KWIC | Draw Desc | Image |

☐ 2. Document ID: AU 9887996 A, DE 19725847 A1, WO 9857992 A2

L2: Entry 2 of 2   File: DWPI   Jan 4, 1999

DERWENT-ACC-NO: 1999-061508
DERWENT-WEEK: 199921
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TITLE: New antibody to FLIP protein - used to suppress inhibition of apoptotic signal transduction by FLIP proteins, to detect FLIP proteins and to screen for substances that activate FLIP expression

INVENTOR: BODMER, J; BURNS, K ; FRENCH, E ; HAHNE, M ; HOFFMANN, K ; IRMLER, M ; RIMOLDI, D ; SCHNEIDER, P ; SCHROETER, M ; STEINER, V ; THOME, M ; TSCHOPP, J ; FRENCH, E L

PRIORITY-DATA: 1997DE-1025847 (June 18, 1997)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 9887996 A	January 4, 1999	N/A	000	C07K016/00
DE 19725847 A1	December 24, 1998	N/A	013	C07K016/00
WO 9857992 A2	December 23, 1998	G	000	C07K016/00

INT-CL (IPC): C07K 16/00; C12N 1/00; C12N 5/10; C12N 15/11; C12N 15/63

| Full | Title | Citation | Front | Review | Classification | Date | Reference |

| KWIC | Draw Desc | Image |

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Terms	Documents
fas and ll	2

Display 200 Documents, starting with Document: 2

Display Format: _____ | Change Format

Search Results -

Terms	Documents
(fas ligand) near100 (gvdh or graft versus host)	1658

Database:
- US Patents Full-Text Database
- US Pre-Grant Publication Full-Text Database
- JPO Abstracts Database
- EPO Abstracts Database
- Derwent World Patents Index
- IBM Technical Disclosure Bulletins

Refine Search: (fas ligand) near100 (gvdh or graft versus host)   Clear

Search History

Today's Date: 5/25/2001

DB Name	Query	Hit Count	Set Name
USPT,PGPB,JPAB,EPAB,DWPI	(fas ligand) near100 (gvdh or graft versus host)	1658	L5
USPT,PGPB,JPAB,EPAB,DWPI	(fas ligand) near200 (gvdh or graft versus host)	1671	L4
USPT,PGPB,JPAB,EPAB,DWPI	(fas ligand) near 200 (gvdh or graft versus host)	365002	L3
DWPI,EPAB,JPAB,PGPB,USPT	fas and l1	2	L2
DWPI,EPAB,JPAB,PGPB,USPT	(FRENCH-L | FRENCH-L-E | VIARD-I | TSCHOPP-J | TSCHOPP-JURG | TSCHOPP-JUERG | TSCHOPP-JUERB-FRIEDRICH | TSCHOPP-JUERG-F | TSCHOPP-JUERG-FRIEDRICH | TSCHOPP-J-F | TSCHOPP-JF | TSCHOPP-JA)!	76	L1

● ● ● ● ● ● ● ● ● ●   Welcome to STN International ● ● ● ● ● ● ● ● ● ●

NEWS 1   Web Page URLs for STN Seminar Schedule - N. America
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NEWS 3 Feb 06 Engineering Information Encompass files have new names
NEWS 4 Feb 16 TOXLINE no longer being updated
NEWS 5 Apr 23 Search Derwent WPINDEX by chemical structure
NEWS 6 Apr 23 PRE-1967 REFERENCES NOW SEARCHABLE IN CAPLUS
AND CA
AND CA
NEWS 7 May 07 DGENE Reload

NEWS EXPRESS May 23 CURRENT WINDOWS VERSION IS V6.0a
CURRENT MACINTOSH VERSION IS V5.0C (ENG) AND V5.0JB (JP),
AND CURRENT DISCOVER FILE IS DATED 06 APRIL 2001
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NEWS PHONE   Direct Dial and Telecommunication Network Access to STN
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=> file medline biosis embase cancerlit scisearch

FILE 'HOME' ENTERED AT 10 02 45 ON 25 MAY 2001

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FILE 'MEDLINE' ENTERED AT 10 03 22 ON 25 MAY 2001

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FILE 'CANCERLIT' ENTERED AT 10 03 22 ON 25 MAY 2001

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                                   ENTRY   SESSION
FULL ESTIMATED COST   0 15   0 15

=> s 'as ligand or

MISSING TERM AFTER LIGAND OR
Operators must be followed by a search term, L-number, or query name

=>

=> s 'fas ligand

L1   11361 FAS LIGAND

=> s l1 and (antibod? or monoclon?)

L2   3148 L1 AND (ANTIBOD? OR MONOCLON?)

=> s l2 and (graft versus host or gvhd)

L3   88 L2 AND (GRAFT VERSUS HOST OR GVHD)

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PROCESSING COMPLETED FOR L3
L4   37 DUP REM L3 (51 DUPLICATES REMOVED)

=> d l4 1-37 bib ab

L4   ANSWER 1 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)
AN   2001 312281 SCISEARCH
GA   The Genuine Article (R) Number 421PM
TI   Transgenic mice ubiquitously expressing human Fas ligand
develop a slight form of graft-versus-host
-like disease
AU   Ma Y H; Fei J; Hu J H; Zhou X G; Xia G H; Guo L H (Reprint)
CS   Chinese Acad Sci, Shanghai Inst Biol Sci, Inst Biochem & Cell Biol,
Shanghai 200031, Peoples R China
CYA   Peoples R China
SO   ACTA PHARMACOLOGICA SINICA, (APR 2001) Vol. 22, No. 4, pp. 311-
319.
Publisher: ACTA PHARMACOLOGICA SINICA, 294 TAI-YUAN ROAD,
SHANGHAI 200031,
PEOPLES R CHINA.
ISSN: 0253-9756
DT   Article, Journal
LA   English
REC   Reference Count: 43
AB   *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB   AIM To construct transgenic mice bearing human Fas
ligand (FasL/CD95L) cDNA, and further explore the physiological
effects of ubiquitous expression of Fast on such animals. METHODS
Transgenic mice were produced by pronuclei microinjection method
Integration and transmission of transgene were identified by nest-PCR and
Southern-blot analysis. Level of Fast mRNA was evaluated by
semi-quantitative RT-PCR analysis. Fast protein was detected by
immunofluorescence analysis. Morphological alterations in tissues were
analyzed by histological examination The percentage of alpha betaT cells
in the spleen was determined by flow cytometry analysis. RESULTS Two
independent founder mice bearing human Fast cDNA under the control of
CMV
promoter were generated healthily. Human Fast was moderately expressed
in
the majority of tissues examined in F1 heterozygotic mice. Although
developing normally, adult transgenic mice exhibited a slight form of
graft-versus-host (GVH)-like disease
characterized by many morphological abnormalities occurring locally in the
spleen, testis, lung and liver. In addition, the percentage of alpha betaT
cells in the spleen was respectively decreased approximately by 32 % and
24 % in two independent transgenic lines, relative to wild-type mice.
CONCLUSION U-biquitous expression of Fas ligand can
lead to slight GVH-like disease.

L4   ANSWER 2 OF 37 EMBASE COPYRIGHT 2001 ELSEVIER SCI
B.V DUPLICATE 1
AN   2000386634 EMBASE
TI   Host T cells resist graft-versus-host
disease mediated by donor leukocyte infusions
AU   Blazar B R ; Lees C J ; Martin P J ; Noelle R J ; Kwon B ; Murphy W ;
Taylor P A
CS   Dr B R Blazar , University of Minnesota Hospital, Box 109 Mayo Building,
420 Southeast Delaware Street, Minneapolis, MN 55455, United States
blaza001@maroon.tc.umn.edu
SO   Journal of Immunology, (1 Nov 2000) 165/9 (4901-4909).
Refs 75
ISSN 0022-1767 CODEN JOIMA3
CY   United States
DT   Journal, Article
FS   026   Immunology, Serology and Transplantation
LA   English
SL   English
AB   Delayed lymphocyte infusions (DLIs) are used to treat relapse occurring
post bone marrow transplantation (BMT) and to increase the donor
chimerism

in recipients receiving nonmyeloablative conditioning As compared with
donor lymphocytes given early post-BMT, DLIs are associated with a reduced
risk of graft-vs-host disease (GVHD). The mechanism(s)
responsible for such resistance have remained incompletely defined We
now
have observed that host T cells present 3 wk after lethal total body
irradiation at the time of DLI, contribute to DLI-GVHD
resistance The infusion of donor splenocytes on day 0, a time when host
resistance The infusion of donor splenocytes on day 0, a time when host
bone marrow (BM)-derived T cells are absent, results in greater expansion
than later post-BMT when host and donor BM-derived T cells coexist
Selective depletion of host T cells with anti-Thy1 allelic mAb increased
in both perforin and Fas ligand but not individually
were highly susceptible to DLI-GVHD Recipients that cannot
produce IFN- gamma were more susceptible to DLI-GVHD whereas
those deficient in IL-12 or p55 TNFRI were not Collectively, these data
regulated DLI-GVHD lethality The conditions by which host T
cells are required for optimal DLI resistance were determined Recipients
unable to express CD28 or 4-1BB were as susceptible to DLI-GVHD
as anti-Thy1 allelic mAb-treated recipients, indicating that CD28 and
4-1BB are critical to DLI-GVHD resistance Recipients deficient
indicate that host T cells, which are capable of generating antidonor CTL
effector cells, are responsible for the impaired ability of DLI to induce
GVHD These same mechanisms may limit the efficacy of DLI in
cancer therapy under some conditions

L4   ANSWER 3 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)
AN   2000 260490 SCISEARCH
GA   The Genuine Article (R) Number 298GC
TI   Blockade of CD134 (OX40)-CD134L interaction ameliorates lethal acute
graft-versus-host disease in a murine model of
allogeneic bone marrow transplantation
AU   Tsukada N; Akiba H; Kobata T (Reprint); Aizawa Y; Yagita H; Okumura K
CS   DOKKYO UNIV, SCH MED, INST MED SCI, DIV IMMUNOL, 880
KITAKOBAYASHI, MIBU,
TOCHIGI 3210293, JAPAN (Reprint); DOKKYO UNIV, SCH MED, INST MED
SCI, DIV
IMMUNOL, MIBU, TOCHIGI 3210293, JAPAN JUNTENDO UNIV, SCH MED,
DEPT
IMMUNOL, TOKYO 113, JAPAN; NIIGATA UNIV, SCH MED, DEPT
INTERNAL MED 1,
NIIGATA, JAPAN; JST, CREST, TOKYO, JAPAN
CYA   JAPAN
SO   BLOOD, (1 APR 2000) Vol. 95, No. 7, pp. 2434-2439
Publisher: AMER SOC HEMATOLOGY, 1200 19TH ST, NW, STE 300,
WASHINGTON, DC
20036-2422
ISSN: 0006-4971
DT   Article, Journal
FS   LIFE, CLIN
LA   English
REC   Reference Count: 47
AB   *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*

AB   Expression of CD134 (OX40) on activated CD4(+) T cells has been
observed in acute graft-versus-host disease
(GVHD) after human and rat allogeneic bone marrow
transplantation (BMT) We investigated the role of interaction between
CD134 and CD134 ligand (CD134L) in a murine model of acute GVHD
by using a newly established monoclonal antibody (mAb)
against murine CD134L. Acute GVHD was induced by transfer of
bone marrow cells and spleen cells into lethally irradiated recipients in
a parent (C57BL/6) to first filial generation (C57BL/6 crossed with DBA/2)
BMT. Administration of anti-CD134L mAb significantly reduced the lethality
of acute GVHD and other manifestations of the disease such as
loss of body weight, hunched posture, diarrhea, and patchy alopecia The
survival rate 80 days after BMT in mice treated with the mAb was about
70%, whereas all mice treated with control antibodies died
within 43 days Histologic examinations revealed that inflammatory changes
in target organs such as the liver, gut, and skin were also ameliorated in
mice treated with the mAb compared with control mice. An in vitro assay of
T-cell proliferation showed a marked hyporesponsiveness to host
alloantigen in samples from mice treated with anti-CD134L mAb In
addition, low levels of interferon gamma and transiently elevated levels
of interleukin 4 and IgE in serum samples were found in mice treated with
anti-CD134L mAb. These results suggest that CD134-CD134L interactions
have

an important role in the pathogenesis of acute GVHD. (Blood
2000 95 2434-2439) (C) 2000 by The American Society of Hematology

L4   ANSWER 4 OF 37 MEDLINE
AN   20038664B4   MEDLINE
DN   PubMed ID: 10898516
TI   Double mutant MRL-lpr/lpr-gld/gld cells fail to trigger lpr graft
      -versus-host disease in syngeneic wild-type recipient
      mice, but can induce wild-type B cells to make autoantibody
AU   Zhu B, Beaudette B C, Rifkin I R, Marshak-Rothstein A
CS   Department of Microbiology, Boston University School of Medicine, MA
      02118, USA
NC   AR35230 (NIAMS)
      DK02597 (NIDDK)
SO   EUROPEAN JOURNAL OF IMMUNOLOGY, (2000 Jun) 30 (6) 1778-84.
      Journal code: EN5. 1273201 ISSN 0014-2980
DT   Journal; Article, (JOURNAL ARTICLE)
LA   English
FS   Priority Journals
EM   20008
ED   Entered STN 20000818
      Last Updated on STN 20000818
      Entered Medline 20000809
AB   Lethally irradiated mice reconstituted with histocompatible stem cells
      from Fas-deficient MRL/lpr mice develop a wasting syndrome reminiscent of
      chronic graft-versus-host disease. However,
      reconstitution with double Fas/Fas ligand
      (FasL)-deficient stem cells does not result in wasting disease,
      demonstrating that FasL expression is an important component of the
      disease double-deficient T cells can induce wild type B cells to make
      autoantibodies. These data indicate that autoantibody production is
      regulated by FasL-expressing T cells, and that Fas-sufficient wild-type B
      cells differ from Fas-deficient lpr cells only with regard to their
      sensitivity to FasL.

L4   ANSWER 5 OF 37 BIOSIS COPYRIGHT 2001 BIOSIS
AN   2001 15298 BIOSIS
DN   PREV200100015298
TI   Fas-mediated cell death in toxic epidermal necrolysis and graft-
      versus-host disease. Potential for therapeutic
      inhibition
AU   French, L E., Tschopp, J (1)
CS   (1) Institute of Biochemistry, Lausanne University, CH-1066, Epalinges,
      Jung Tschopp@ib unil ch Switzerland
SO   Schweizerische Medizinische Wochenschrift, (4 November, 2000) Vol
      130,
      No 44, pp 1656-1661, print
      ISSN 0036-7672
DT   Article
LA   English
AB   Death receptors are a growing family of transmembrane proteins which can
      detect the presence of specific extracellular death signals and rapidly
      trigger cellular destruction by apoptosis. The best studied to date is Fas
      (CD95). Expression and signalling by Fas and its ligand (FasL, CD95L) is a
      tightly regulated process essential for key physiological functions in a
      variety of organs, including the maintenance of immune homeostasis
      Recently, strong evidence has shown that dysregulation of Fas expression
      and/or signalling contributes to the pathogenesis of toxic epidermal
      necrolysis and acute graft-versus-host
      disease. With these new developments, strategies for modulating the
      function of Fas signalling have emerged and opened up novel therapeutic
      possibilities. Specific blockade of Fas, for example with intravenous
      immunoglobulin preparations containing specific anti-Fas
      antibodies, has shown great promise in the treatment of toxic
      epidermal necrolysis and may also be useful in the treatment of acute
      graft versus-host disease Further
      developments in this field may have important clinical implications for
      the treatment of such diseases

L4   ANSWER 6 OF 37 EMBASE COPYRIGHT 2001 ELSEVIER SCI B V
AN   2001018875 EMBASE
TI   Derangement of apoptosis-related lymphocyte homeostasis in systemic
      sclerosis
AU   Stummvoll G H, Aringer M, Smolen J S, Koller M, Kiener H P, Steiner
      C W, Bohle B, Knobler R, Graninger W B
CS   W B Graninger, Department of Rheumatology, Internal Medicine III,
      University of Vienna, Währinger Gürtel 18-20, A-1090 Vienna, Austria
SO   Rheumatology, (2000) 39/12 (1341-1350)
      ISSN 1462-0324 CODEN RUMAFK
CY   United Kingdom
DT   Journal; Article
FS   005   General Pathology and Pathological Anatomy
      026   Immunology, Serology and Transplantation
      031   Arthritis and Rheumatism
      037   Drug Literature Index
LA   English
SL   English
AB   Objectives Both increased and decreased apoptosis may be involved in
      generating autoimmunity. This study addressed the question of whether
      apoptosis and apoptosis-regulating proteins are altered in systemic
      sclerosis (SSc). Patients and methods Peripheral lymphocytes of 39 Ssc
      patients and 47 healthy control persons were studied for apoptosis, Bcl-2
      and Bax levels, expression of Fas (CD95) and activation markers (CD25,
      HLA-DR) as determined by fluorocytometry Serum Fas and Fas
      ligand were measured by ELISA Results SSc lymphocytes (mainly
      CD4(+)) expressed increased amounts of Bcl-2, while Bax was not elevated
      Apoptosis rates of SSc lymphocytes were increased in unsupplemented
      medium, but returned to normal in the presence of autologous plasma SSc
      patients had increased percentages of activated and CD95(+) lymphocytes
      and elevated soluble Fas and soluble FasL levels in serum. Activating
      anti-CD95 antibodies further increased the apoptosis rate
      Conclusions Increased in vitro apoptosis, elevated lymphocytic Bcl-2
      content and the increased number of Fas-positive T cells are not specific
      for peripheral blood from SSc patients, but indicate deregulation of
      lymphocyte homeostasis in this disease

L4   ANSWER 7 OF 37 MEDLINE
AN   2001102227 MEDLINE
DN   20408065 PubMed ID: 10953977
TI   A metalloproteinase inhibitor prevents acute graft-
      versus-host disease while preserving the
      graft-versus-leukaemia effect of allogeneic bone marrow transplantation
AU   Hattori K, Hirano T, Oshimi K, Yagita H, Okumura K
CS   Department of Internal Medicine, Juntendo University School of Medicine,
      Tokyo, Japan
SO   LEUKEMIA AND LYMPHOMA, (2000 Aug) 38 (5-6) 553-61
      Journal code: BNQ. ISSN 1042-8194.
CY   Switzerland
DT   Journal, Article, (JOURNAL ARTICLE)
LA   English
FS   Priority Journals
EM   200101
ED   Entered STN 20010322
      Last Updated on STN 20010322
      Entered PubMed 20010123
      Entered Medline 20010126
AB   Tumor necrosis factor (TNF) and Fas ligand (FasL) have
      been implicated in the pathogenesis of graft-versus-
      host disease (GVHD). Several recent studies have shown
      that some metalloproteinase mediates TNF-alpha and FasL processing. We
      examined the ameliorating effect of a hydroxamic acid-based
      metalloproteinase inhibitor (KB-R7785) that inhibits TNF-alpha and FasL
      release in a lethal acuteGVHD model in mice. The ameliorating effect of
      KB-R7785 was superior to that of anti-TNF-alpha antibody. We
      also examined the effect of KB-R7785, which we previously demonstrated a
      potent ameliorating effect on acute GVHD, on
      graft-versus-leukemia (GVL) effect of allogeneic bone marrow
      transplantation (BMT). Administration of KB-R7785 without bone marrow
      cells and spleen cells (BMS), significantly prolonged the survival of
      IgE-producing B53 hybridoma cell-inoculated (C57BL/6 x BALB/c) F1 (CBF1)
      mice by inhibiting the infiltration of B53 cells into the liver and
      spleen. Transplantation of B6 BMS without KB-R7785 resulted in the death
      of most recipients due to acute GVHD while efficiently
      eliminating B53 cells. Administration of KB-R7785 along with B6 BMS
      resulted in 50% survival of B53-inoculated CBF1 mice over 50 days without
      histological manifestations of acute GVHD or residual B53 cells
      These results suggest that KB-R7785 could be a potent therapeutic agent
      for GVHD, and indicate the beneficial effects of KB-R7785 that

inhibit tumor infiltration and prevent acute GVHD while
preserving the GVL effect of allogeneic BMT

L4   ANSWER 8 OF 37 EMBASE COPYRIGHT 2001 ELSEVIER SCI B V
AN   2000418994 EMBASE
TI   Anti-third party CD8+ CTLs as potent veto cells. Coexpression of CD8 and
      FasL is a prerequisite
AU   Reich-Zeliger S, Zhao Y, Krauthgamer R, Bachar-Lustig E, Reisner Y
CS   Y. Reisner, Department of Immunology, Weizmann Institute of Science,
      Rehovot 76100, Israel yair.reisner@weizmann.ac.il
SO   Immunity, (2000) 13/4 (507-515)
      ISSN 1074-7613 CODEN IUNIEH
      Refs. 58
CY   United States
DT   Journal; Article
FS   026   Immunology, Serology and Transplantation
LA   English
SL   English
AB   Several bone marrow cells and lymphocyte subpopulations, known as 'veto
      cells,' were shown to induce transplantation tolerance across major
      histocompatibility antigens Recently, it has been suggested that
      anti-third party CTLs depleted of alloreactivity are endowed with marked
      veto activity and therefore might potentially facilitate bone marrow
      allografting without graft versus host
      disease (GVHD) The veto mechanism is still obscure While early
      studies emphasized the role of CD8-mediated apoptosis, more recent
      evidence indicates a role for Fas-FasL. In the present study we show, by
      using blocking anti-CD8 antibody, by generating CTLs from FasL
      or perforin mutated mice, and by gene transfer of FasL, that the veto
      activity of anti-third party CD8+ CTLs is dependent upon the simultaneous
      expression of both CD8 and FasL

L4   ANSWER 9 OF 37 MEDLINE
AN   2000356783 MEDLINE
DN   20356783 PubMed ID: 10901605
TI   T-cell co-signaling molecules in graft-versus-
      host disease
AU   Tanaka J, Asaka M, Imamura M
CS   Department of Haematology and Oncology, Hokkaido University School of
      Medicine, Sapporo, Japan
SO   ANNALS OF HEMATOLOGY, (2000 Jun) 79 (6) 283-90 Ref. 80
      Journal code: A2P. 9107334 ISSN 0939-5555
CY   GERMANY Germany, Federal Republic of
DT   Journal, Article, (JOURNAL ARTICLE)
      General Review, (REVIEW)
      (REVIEW, TUTORIAL)
LA   English
FS   Priority Journals
EM   200007
ED   Entered STN 20000811
      Last Updated on STN 20000811
      Entered Medline 20000728
AB   Allogeneic stem cell transplantation (allo SCT) is now frequently
      performed for the treatment of haematological malignancies and aplastic
      anaemia However, graft-versus-host disease
      (GVHD) is still the major complication after allo SCT, producing
      immune deficiency, infection, organ damage and, occasionally, patient
      death. The antigen-specific signal mediated by the T-cell receptor (TCR)
      is essential for activation of T-cells; however, additional co-stimulatory
      signals are required for complete T-cell activation. Therefore, blocking
      strategies of co-stimulatory signals have been evaluated as targets of
      therapeutic intervention for GVHD after allo SCT. In a mouse
      bone-marrow transplantation (BMT) model, the administration of CTLA4-Ig,
      which blocks the interaction of CD28 on T-cells and B7 molecules on
      antigen-presenting cells (APCs), can prolong survival of allo BMT
      recipients, although this effect was not complete. In addition, the
      anti-CD40L (CD154) monoclonal antibody (mAb), which
      can interfere with the interaction of CD154 on T-cells and CD40 on APCs,
      can induce long-term graft survival in the murine model. Combined
      administration of CTLA4-Ig and anti-CD40L mAb can prevent allograft
      rejection in primates. Therefore, it seems the most powerful method to
      prevent the alloimmune response in vivo The Fas/Fas
      ligand pathway is also involved in pathogenesis of GVHD
      Anti-FasL mAb can reduce the mortality of GVHD and improve
      intestinal lesions. Recently, it was reported that donor bone marrow
      treated ex vivo using CTLA4-Ig reconstituted haematopoiesis in vivo with a

DUPLICATE 2

DUPLICATE 3

DUPLICATE 4

relatively low risk of GVHD in human allo BMI Therefore, selective blocking strategies for T-cell co signaling might be useful for the prevention of GVHD in human allo SCT

B V DUPLICATE 5
AN 2000091287 EMBASE
TI Effect of a matrix metalloprotease inhibitor on host resistance against Listeria monocytogenes infection
AU Yamada K , Yoshino K , Sekikawa K , Madarame H , Yagita H , Nakane A
CS A Nakane, Department of Bacteriology, Hirosaki University Sch of Medicine, Zaifu-cho 5, Hirosaki, Aomori 036-8562, Japan
     a27k03nd@cc.hirosaki-u.ac.jp
SO FEMS Immunology and Medical Microbiology (4 Nov 2000) 29(3 (187-194)
     Refs 40
     ISSN 0928-8244 CODEN FIMIEV
PUI S 0928-8244(00)00204-2
CY Netherlands
DT Journal, Article
FS 004 Microbiology
     037 Drug Literature Index
LA English
AB Hydroxy acid-based matrix metalloproteinase (MMP) inhibitors have been shown to inhibit tumor infiltration and growth, endotoxin shock, and acute graft-versus-host disease Blockade of the release of soluble tumor necrosis factor- alpha (TNF- alpha) and CD95 ligand (CD95L, FasL) from cell-associated forms is reportedly involved in the mechanism of the drug effect We investigated the effect of a MMP inhibitor, KB-R7785, on host resistance against Listeria monocytogenes infection , in which TNF- alpha is essentially required for the defense in mice The administration of KB-R7785 exacerbated listeriosis, while the drug prevented lethal shock induced by lipopolysaccharide and D-galactosamine KB-R7785 inhibited soluble TNF- alpha production in spleen cell cultures stimulated by heat-killed L monocytogenes and the drug treatment reduced serum TNF- alpha levels in infected mice, whereas the compound was ineffective on the the modulation of interferon- gamma and interleukin-10 production The effect of KB-R7785 was considered to be dependent on TNF- alpha because the drug failed to affect L monocytogenes infection in anti-TNF- alpha monoclonal antibody-treated mice and TNF- alpha knockout mice Anti-CD95L monoclonal antibody was also ineffective on the infection These results suggest that induction of infectious diseases, to which TNF- alpha is critical in host resistance, should be considered in MMP inhibitor-treated hosts (C) 2000 Federation of European Microbiological Societies

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L4 ANSWER 11 OF 37 MEDLINE   DUPLICATE 6
AN 2000429103 MEDLINE
DN 20409099 PubMed ID 10951228
TI Death receptors in cutaneous biology and disease
AU Wehrli P, Viard I, Bullani R, Tschopp J, French L E
CS Department of Dermatology, Geneva University Medical School, Geneva, Switzerland
SO JOURNAL OF INVESTIGATIVE DERMATOLOGY. (2000 Aug) 115 (2) 141-8 Ref 87
     Journal code IHZ, 0426720 ISSN 0022-202X
CY United States
DT Journal, Article, (JOURNAL ARTICLE)
     General Review, (REVIEW)
     (REVIEW, TUTORIAL)
LA English
FS Priority Journals
EM 200009
ED Entered STN 20000922
     Last Updated on STN 20000922
     Entered Medline 20000914
AB Death receptors are a growing family of transmembrane proteins that can detect the presence of specific extracellular death signals and rapidly trigger cellular destruction by apoptosis Expression and signaling by death receptors and their respective ligands is a tightly regulated process essential for key physiologic functions in a variety of organs, including the skin Several death receptors and ligands, Fas and Fas ligand being the most important to date, are expressed in the skin and have proven to be essential in contributing to its functional integrity Recent evidence has shown that Fas-induced keratinocyte apoptosis in response to ultraviolet light prevents the

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accumulation of pro-carcinogenic p53 mutations by deleting ultraviolet-mutated keratinocytes Further, more, there is strong evidence that dysregulation of Fas expression and/or signaling contributes to the pathogenesis of toxic epidermal necrolysis, acute cutaneous graft versus host disease contact hypersensitivity and melanoma metastasis With these new developments, strategies for modulating the function of death receptor signaling pathways have emerged and provided novel therapeutic possibilities Specific blockade of Fas, for example with intravenous immunoglobulin preparations that contain specific anti-Fas antibodies, has shown great promise in the treatment of toxic epidermal necrolysis and may also be useful in the treatment acute graft versus host disease Likewise, induction of death signaling by ultraviolet light can lead to hapten-specific tolerance, and gene transfer of Fas ligand to dendritic cells can be used to induce antigen-specific tolerance by deleting antigen-specific T cells Further developments in this field may have important clinical implications in cutaneous disease

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L4 ANSWER 12 OF 37 MEDLINE   DUPLICATE 7
AN 2000118144 MEDLINE
DN 20118144 PubMed ID 10651946
TI Graft-versus-host-disease-associated donor cell engraftment in an F1 hybrid model is dependent upon the Fas pathway,
AU Iwasaki T, Hamano T, Saheki K, Kuriowa T, Kataoka Y, Takemoto Y, Ogata A,
     Fujimoto J, Kakishita E
CS The Second Department of internal Medicine, Hyogo College of Medicine, Nishinomiya, Hyogo, Japan
SO IMMUNOLOGY (2000 Jan) 99 (1) 94-100.
     Journal code, GH7, 0374672 ISSN 0019-2805
CY ENGLAND United Kingdom
DT Journal, Article, (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200002
ED Entered STN 20000218
     Last Updated on STN 20000218
     Entered Medline 20000210
AB The graft-versus-host disease (GVHD) generated in BDF1 mice by the injection of spleen cells from the C57BL/6 parental strain induces a direct cell-mediated attack on host lymphohaematopoietic populations, resulting in the reconstitution of the host with donor cells We examined Fas-Fas ligand (FasL) interactions in donor and host haematopoietic cells over a prolonged period of parental-induced GVHD Fas expression on bone marrow cells of both donor and host origin increased at 2 weeks Host cell incubation with anti-Fas antibody induced apoptosis, and the number of haematopoietic progenitor cells decreased Fas-induced apoptosis by the repopulating donor cells, however, did not increase until 12 weeks, when more than 90% of the cells were donor cells The expression of various cytokines, such as interferon-gamma (IFN-gamma) and tumour necrosis factor-alpha (TNF-alpha), and FasL gene expression in the bone marrow increased concomitantly To examine directly whether FasL has a major role in the development of donor cell engraftment, FasL-deficient (gld) mice were used as donors Injection of B6/gld spleen cells induced significantly less host lymphohaematopoietic depletion, resulting in a failure of donor cell engraftment Furthermore, injection of IFN-gamma gene knockout (gko) B6 spleen cells failed to augment Fas and FasL expression in recipient mice, resulting in a failure of donor cell engraftment This suggests that the induction of apoptosis by Fas-FasL interactions in host cells may contribute to a reconstitution of the host with donor cells and that donor-derived IFN-gamma plays a significant role for Fas-FasL interactions in host cells during parental-induced GVHD

L4 ANSWER 13 OF 37 BIOSIS COPYRIGHT 2001 BIOSIS DUPLICATE 8
AN 2000 454060 BIOSIS
DN PREV200000454060
TI Role of perforin in controlling B-cell hyperactivity and humoral autoimmunity
AU Shustov, Andrei, Luzina, Irina, Nguyen, Phuong, Papadimitriou, John C.,
     Handwerger, Barry, Eikon, Keith B., Via, Charles S. (1)
CS (1) Division of Rheumatology and Clinical Immunology, University of Maryland School of Medicine, 10 S. Pine Street, MSTF 8-34, Baltimore, MD, 21201 USA

SO Journal of Clinical Investigation, (September, 2000) Vol 106 No 6, pp R39-R47 print
     ISSN 0021-9738
DT Article
LA English
SL English
AB To determine the role of perforin-mediated cytotoxic T lymphocyte (CTL) effector function in immune regulation, we studied a well-characterized mouse model of graft-versus-host disease (
GVHD) Induction of acute GVHD using perforin-deficient donor T cells (pfp/pfpdarvF1) initially resulted in features of acute GVHD, e.g., engraftment of both donor CD4+ and CD8+ T cells, upregulation of Fas and FasL, production of antihost CTL, and secretion of both Th1 and Th2 cytokines Despite fully eliminate host B cells, and, by 4 weeks of disease cytokine production in pfpfwdarvF1 mice had polarized to a Th2 response Pfpfwdarvf1 mice eventually developed features of chronic GVHD, such as increased numbers of B cells, persistence of donor CD4 T cells, autoantibody production, and lupuslike renal disease We conclude that in the setting of B- and T-cell activation, perforin plays an important immunoregulatory role in the prevention of humoral autoimmunity through the elimination of both autoreactive B cells and ag-specific T cells Moreover, an ineffective initial CTL response can evolve into a persistent antibody-mediated response and, with it, the potential for sustained humoral autoimmunity

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L4 ANSWER 14 OF 37 MEDLINE
AN 1999218481 MEDLINE
DN 99218481 PubMed ID 10201983
TI Aberrant CD3- and CD28-mediated signaling events in cord blood T cells are associated with dysfunctional regulation of Fas ligand
     -mediated cytotoxicity
AU Sato K, Nagayama H, Takahashi T A
CS Department of Cell Processing, Institute of Medical Science, University of Tokyo, Japan.
SO JOURNAL OF IMMUNOLOGY, (1999 Apr 15) 162 (8) 4464-71
     Journal code IFB, 2985117R ISSN 0022-1767
CY United States
DT Journal, Article, (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals, Priority Journals
EM 199905
ED Entered STN 19990517
     Last Updated on STN 19990517
     Entered Medline 19990506
AB There have been numerous reports of decreased acute and chronic graft-vs-host disease (GVHD) in patients receiving HLA-matched or HLA-disparate umbilical cord transplants However, little is known about the mechanisms underlying the low incidence of GVHD in umbilical cord blood transplantation (CBT) In this study, we examined CD3- and CD28-mediated functional properties and signaling events in CB T cells (CBTCs) Dual stimulation of peripheral blood TCs (PBTCs) and bone marrow TCs (BMTCs) with mAbs to CD3- and CD28-induced expressions of Fas ligand (FasL), as well as CD25 and CD154 (CD40L), whereas defective induction of these activation-associated cell surface molecules were observed in CBTCs Engagement of both CD3 and CD28 induced FasL-mediated cytotoxicity in peripheral blood TCs (PBTCs), but not CBTCs, however, both of these tissue sources possess intrinsically similar proliferative responsiveness Analysis of CD3- and CD28-induced signal transduction revealed a deficiency in signaling events that involved repressed tyrosine phosphorylation and enzymatic activities of a family of mitogen-activated protein kinases, extracellular signal-regulated kinase 2, stress-activated protein kinase/c-jun N-terminal kinase (SAPK/JNK), and p38mapk, as well as p56ick and ZAP-70 in CBTCs compared with those in PBTCs These results suggest that CD3- and CD28-mediated signaling events blockade in CBTCs may be responsible for dysfunction of FasL-mediated cytotoxicity and lead to the low incidence of severe GVHD in CBT

L4 ANSWER 15 OF 37 MEDLINE   DUPLICATE 9
AN 1999211960 MEDLINE
DN 99211960 PubMed ID 10194454
TI Graft-versus-leukemia effect and graft-versus-host disease can be differentiated by cytotoxic mechanisms in a

murine model of allogeneic bone marrow transplantation

AU Tsukada N, Kobata T, Aizawa Y, Yagita H, Okumura K
CS Department of Immunology, Juntendo University School of Medicine, Tokyo, Japan
SO BLOOD, (1999 Apr 15) 93 (8) 2738-47.
Journal code: A8G; 7603509 ISSN 0006-4971
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus; Journals; Priority Journals
EM 19990S
ED Entered STN: 19990517
Last Updated on STN: 19990503
AB Allogeneic bone marrow transplantation (allo-BMT) is associated with both graft-versus-host disease (GVHD) and graft-versus-leukemia (GVL) effect. In the present study, we examined the contribution of cytotoxic effector mechanisms, which are mediated by tumor necrosis factor-alpha (TNF-alpha), Fas ligand (FasL), perforin, to GVHD and GVL effect in a murine BMT model. Bone marrow cells plus spleen cells (BMS) from wild-type, FasL-defective, or perforin-deficient donors were transferred into lethally irradiated recipients in the parent (C57BL/6) to F1 (C57BL/6 x DBA/2) BMT model with or without prior inoculation of DBA/2 leukemia L1210 or P815 mast cytoma cells. The effect of anti-TNF-alpha antibody administration was also examined. Whereas the defect or blockade of each cytotoxic pathway could ameliorate lethal acute GVHD, the GVL effect was differentially affected. The wild-type BMS recipients died of acute GVHD within 50 days without residual leukemia cells. The FasL-defective BMS recipients showed 60%~ survival over 80 days with acute GVHD or residual leukemia cells. Administration of anti-TNF-alpha antibody resulted in early leukemia relapse and the recipients died within 25 days with massive leukemia infiltration in the liver. The perforin-deficient BMS recipients died within 60 days with residual leukemia cells. These results suggest that blockade of the Fas/FasL pathway could be used for ameliorating GVHD without impairing GVL effect in allo-BMT.

L4 ANSWER 16 OF 37 MEDLINE
AN 200000330 MEDLINE
DN 20000330 PubMed ID 10532530
TI Analysis of Fas system in pulmonary injury of graft-versus-host disease after rat intestinal transplantation
AU Hiroyasu S, Shiraishi M, Koji T, Mamadi T, Sugawa H, Tomori H, Muto Y
CS The First Department of Surgery, University of the Ryukyus, School of Medicine, Okinawa, Japan
SO TRANSPLANTATION, (1999 Oct 15) 68 (7) 933-8.
Journal code: WEJ; 0132144 ISSN 0041-1337.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199911
ED Entered STN: 20000111
Last Updated on STN: 20000111
AB The lung is one of the primary targets of acute graft-versus-host disease (GVHD), which is the principal complication that occurs after allogeneic transplantation. The purpose of this study is to investigate the involvement of Fas/Fas ligand system in pulmonary injury after rat semi-allogeneic intestinal transplantation. The lungs were serially harvested from LEW x BN F1(LBNF1) recipients of either LEW heterotopic intestinal allografts or LBNF-1 isografts, on days 1, 3, 5, 9, and 13 posttransplant. In light microscopy, pulmonary injury became apparent on day 13 in the allogeneic combination, showing a thickening of the alveolar septa. The incidence of apoptosis, examined by terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate (dUTP) biotin nick end-labeling, was observed to increase steadily in the alveolar cells accompanied by a progression of GVHD. In an immunohistochemical study, Fas was constitutively expressed most extensively on alveolar cells, in addition to leukocytes, although Fas ligand was expressed most extensively on day 9. The immunoreactivity of both Fas and Fas ligand were observed in alveolar cells, in addition to leukocytes. An analysis by

reverse transcription polymerase chain reaction also revealed that the expression of Fas mRNA was constitutive without any significant change, although that of Fas ligand mRNA increased substantially and peaked on day 9, which was significant compared to the isogeneic combination. In conclusion, transcriptionally up-regulated Fas ligand and increased number of apoptosis suggests that the Fas system may play a role in the pathophysiology of GVHD-induced pulmonary injury.

L4 ANSWER 17 OF 37 MEDLINE   DUPLICATE 10
AN 99289372 MEDLINE
DN 99289372 PubMed ID 10360966
TI Therapeutic effect of an anti-Fas ligand mAb on lethal graft-versus-host disease
AU Miwa K, Hashimoto H, Yatomi T, Nakamura N, Nagata S, Suda T
CS Department of Molecular Biology, Osaka Bioscience Institute, Osaka 565-0874, Japan
SO INTERNATIONAL IMMUNOLOGY, (1999 Jun) 11 (6) 925-31.
Journal code: AY5; 8916182 ISSN: 0953-8178
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199908
ED Entered STN: 19990820
Last Updated on STN 19990820
AB Several anti-Fas ligand (FasL) inhibitory mAb (FLIM) were raised and characterized in this study. One, FLIM58, showed more potent neutralizing activity than Fas-Fc, the previously established artificial neutralizing agent for FasL. Several murine models of acute graft-versus-host disease (GVHD) after allogeneic bone marrow transplantation have been used to show that both FasL and perforin, the major effector molecules of cytotoxic T lymphocytes, are involved in this disease. In our GVHD model, FasL, rather than perforin was associated with lethality. Administration of FLIM58 or Fas-Fc reduced the weight loss and mortality caused by GVHD, although other signs of GVHD, such as skin lesions, lymphoid hypoplasia and mononuclear cell infiltration in the liver, did not improve significantly. FLIM58 was more effective than Fas-Fc in reducing mortality. Our results demonstrated that neutralizing agents for FasL are therapeutic for lethal GVHD.

L4 ANSWER 18 OF 37 EMBASE COPYRIGHT 2001 ELSEVIER SCI
B V DUPLICATE 11
AN 1999380003 EMBASE
TI Graft-versus-host disease-like immunophenotype and apoptotic keratinocyte death in paraneoplastic pemphigus.
AU Reich K.; Binck U.; Letschert M.; Blaschke V.; Danes K.; Braess J.; Wormann B.; Runger T.M.; Neumann C
CS K. Reich, Department of Dermatology, Georg-August-University, von Siebold Strasse 3, D-37075 Gottingen, Germany. kreich@gwdg.de
SO British Journal of Dermatology, (1999) 141/4 (739-746)
Refs: 34
ISSN: 0007-0963 CODEN: BJDEAZ
CY United Kingdom
DT Journal; Article
SL English
FS 013 Dermatology and Venereology
037 Drug Literature Index
LA English
AB Paraneoplastic pemphigus (PP) is an autoimmune disease which is frequently associated with non-Hodgkin's lymphoma. Autoantibodies against components of the cytoplasmic plaque of epithelial desmosomes are usually present in the sera and are believed to play a major pathogenic part in acantholysis and suprabasal epidermal blistering. However, another typical histological feature of PP, interface dermatitis with keratinocyte dyskeratosis, is shared with skin diseases that involve epithelial damage mediated by T cells. Here, we present the detailed characterization of the cutaneous T-cell response in a patient with PP and demonstrate a selective epidermal accumulation of activated CD8+T cells together with an increased local production of interferon- gamma and tumour necrosis factor- alpha, and a strong expression of HLA-DR and ICAM-1 on keratinocytes. Apoptosis was identified as a key mechanism of keratinocyte death, and appeared

independent of the FAS/FAS ligand (FAS-L) pathway as epidermal expression of FAS was not increased compared with normal skin and FAS-L was undetectable on the protein and mRNA level. Triple therapy with high-close corticosteroids, cyclophosphamide and intravenous immunoglobulins reduced levels of pemphigus-like autoantibodies and reversed the cutaneous inflammatory reaction leading to long-standing clinical remission. Our findings support the concept of a major contribution of cytotoxic T lymphocytes to the immunopathology of paraneoplastic pemphigus.

L4 ANSWER 19 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)
AN 1999 556331 SCISEARCH
GA The Genuine Article (R) Number: 215VW
TI An in vitro model of allogeneic stimulation of cord blood - Induction of graft-versus-host disease
AU ElGhalbzouri A, Drenou B, Blancheteau V, Choqueux C, Fauchet R, Charron D
Mooney N (Reprint)
CS INST BIOMED CORDELIERS, INSERM, U396, IMMUNOGENET MOL LAB, INSERM, U396, IMMUNOGENET MOL LAB, 15 RUE ECOLE MED, F-75006 PARIS, FRANCE (Reprint), INST BIOMED CORDELIERS, PONTCHAILLOU LAB HEMATOL & IMMUNOL, F-35033 RENNES 9, FRANCE
CYA FRANCE
SO HUMAN IMMUNOLOGY, (JUL 1999) Vol 60, No. 7, pp 598-607
Publisher: ELSEVIER SCIENCE INC, 655 AVENUE OF THE AMERICAS, NEW YORK, NY 10010
ISSN 0198-8859
DT Article; Journal
FS LIFE
LA English
REC Reference Count 30
"ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS"
AB Cord blood is increasingly used in transplantation as it is a readily available source of progenitor cells and is reputed to generate less severe graft-versus-host disease (GVHD) than adult bone marrow. We have compared apoptosis of cord blood lymphocytes (CB) and adult lymphocytes (PBMC) after stimulation via HLA-class I, HLA class II or CD3 in order to reproduce in vitro some of the stimuli occurring after alloltransplantation. CB spontaneously apoptose more than PBMC ex vivo, stimulation via HLA class I dramatically increased CB apoptosis without altering viability of PBMC. Expression of Fas was markedly lower on CB than on PBMC and this difference was maintained even after activation. Fas ligand Twas expressed in CB and in PBMC. CB were activated via either HLA class I or class II molecules although proliferation was not observed. Only photbol ester pre-activation allowed Fas to subsequently induce a death signal. Proliferation of PBMC via CD3 led to enhanced Fas signals. CB therefore differ from PBMC with regard to both spontaneous and activation induced apoptosis and either allo- or CD3 mediated stimulation. Finally, the apoptosis of CB via HLA-class I could have an important role in the moderation of graft-versus-hose disease [Human Immunology 60, 598-607 (1999)] (C) American Society for Histocompatibility and Immunogenetics, 1999 Published by Elsevier Science Inc.

L4 ANSWER 20 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)
AN 1999 358258 SCISEARCH
GA The Genuine Article (R) Number: 192CP
TI A metalloproteinase inhibitor prevents acute graft-versus-host disease while preserving the graft-versus-leukaemia effect of allogeneic bone marrow transplantation
AU Hattori K, Hirano T (Reprint), Miyajima H, Yanakawa N, Ikeda S, Yoshino K, Tateno M, Oshimi K, Kayagaki N, Yagita H, Okumura K
CS JUNTENDO UNIV, SCH MED, DEPT INTERNAL MED, DIV HAEMATOL, BUNKYO KU, 2-1-1 HONGO, TOKYO 113, JAPAN (Reprint), JUNTENDO UNIV, SCH MED, DEPT INTERNAL MED, DIV HAEMATOL, BUNKYO KU, TOKYO 113, JAPAN, JUNTENDO UNIV, SCH MED, DIV PATHOBIOL, TOKYO 113, JAPAN, JUNTENDO UNIV, SCH MED, DEPT IMMUNOL

TOKYO 113 JAPAN KANEBO LTD, NEW DRUG DISCOVERY RES LAB,
OSAKA 534
JAPAN SAPPORO CITY GEN HOSP DEPT PATHOL, SAPPORO,
HOKKAIDO, JAPAN, JAPAN
SCI & TECHNOL CORP CREST, TOKYO, JAPAN

CYA JAPAN
SO BRITISH JOURNAL OF HAEMATOLOGY, (APR 1999) Vol. 105, No. 1, pp.
303-312
Publisher BLACKWELL SCIENCE LTD, P O BOX 88, OSNEY MEAD,
OXFORD OX2 ONE
OXON, ENGLAND
ISSN 0007-1048
DT Article; Journal
FS LIFE, CLIN
LA English
REC Reference Count 39

AB We examined the effect of a hydroxamic acid-based matrix
metalloproteinase inhibitor (KB-R7785), which we previously demonstrated
to have a potent ameliorating effect on acute graft
versus-host disease (GVHD), and on the
graft-versus-leukaemia (GVL) effect of allogeneic bone marrow
transplantation (BMT). KB-R7785 was administered to (C57BL/6 x BALB/c)
CBF1) mice that had been inoculated with IgE-producing B53 hybridoma
cells of BALB/c origin as a model tumour, along with or without
transplantation of C57BL/6 (B6) bone marrow cells and spleen cells (BMS).
Administration of KB-R7785 without BMS significantly prolonged the
survival of B53-inoculated CBF1 mice by inhibiting the infiltration of B53
cells into the liver and spleen. Transplantation of B6 BMS without
KB-R7785 resulted in the death of most recipients due to acute
GVHD while efficiently eliminating B53 cells. Administration of
KB-R7785 along with B6 BMS resulted in a 50% survival of B53-inoculated
CBF1 mice over 50 d without histological manifestations of acute
GVHD or residual B53 cells. These results indicate the beneficial
effects of KB-R7785 that inhibit tumour infiltration and prevent acute
GVHD while preserving the GVL effect of allogeneic BMT

L4 ANSWER 21 OF 37 MEDLINE
AN 1999332261 MEDLINE
DN 99332261 PubMed ID 10403735
TI intestinal crypt cell apoptosis in murine acute graft
versus host disease is mediated by tumour necrosis
factor alpha and not by the FasL-Fas interaction: effect of pentoxifylline
on the development of mucosal atrophy
AU Stuber E, Buschenfeld A, von Freier A, Arendt T, Folsch U R
CS 1 Medizinische Universitatsklinik, Department of Internal Medicine,
Christian-Albrechts-Universitat, Kiel, Germany
SO GUT, (1999 Aug) 45 (2) 229-35
Journal code FVT, 2985108R ISSN 0017-5749
CY ENGLAND, United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
EM English
EM 199909
ED Entered STN 19990925
Last Updated on STN 19990925
Entered Medline 19990914
AB BACKGROUND Murine T cell mediated acute semiallogeneic graft
versus host disease (GVHD) is characterised by
lymphocytic infiltrates, crypt hyperplasia, and villous atrophy. It has
been shown that programmed cell death (apoptosis) of the crypt epithelium
takes place during the intestinal manifestation of acute GVHD
AIMS To investigate which of the two most investigated inductors of
apoptosis (Fas ligand (FasL) and tumour necrosis
factor alpha (TNF-alpha)) is responsible for the induction of apoptosis in
this animal model METHODS Animals undergoing acute semiallogeneic
GvH

reaction were treated with neutralising anti-TNF-alpha, two different
anti-FasL antibodies, or pentoxyfylline RESULTS Anti-TNF-alpha
application inhibited the appearance of apoptotic cells in the intestinal
mucosa, whereas anti-FasL antibodies had no influence on mucosal
apoptosis. In addition, the transfer of FasL deficient (gld) donor
lymphocytes still induced crypt cell apoptosis, villous atrophy, and crypt
hyperplasia Furthermore, when the animals were treated with
pentoxifylline, a known inhibitor of TNF-alpha secretion in vitro and in
vivo, there was significant normalisation of the intestinal morphology
accompanied by inhibition of epithelial apoptosis CONCLUSIONS The
FasL-Fas interaction is not involved in the induction of apoptosis during
acute GVHD However, neutralisation of TNF-alpha by an
antibody or by pentoxyfylline inhibits the occurrence of apoptosis
and of mucosal atrophy in this animal model. These results have
implications for the treatment of immunologically mediated human atrophic
gut diseases for example, diet refractory cases of coeliac disease

L4 ANSWER 22 OF 37 MEDLINE   DUPLICATE 13
AN 1999239157 MEDLINE
DN 99239157 PubMed ID 10222651
TI Therapeutic strategy for post-transfusion graft-vs.-host disease.
AU Saigo K, Ryo R
CS Blood Transfusion Division, Kobe University Hospital, Japan
saigo@med kobe-u ac.jp
SO INTERNATIONAL JOURNAL OF HEMATOLOGY, (1999 Apr) 69 (3) 147-51
Ref 29
Journal code A7F; 9111627 ISSN 0925-5710
CY Ireland
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW; TUTORIAL)
LA English
FS Priority Journals
EM 199905
ED Entered Medline 19990601
Last Updated on STN 19990601
AB An effective treatment for post-transfusion graft-vs.-host disease (PT-
GVHD), a fatal complication of blood transfusion, has not yet been
identified. In this review, we propose a treatment for PT-GVHD
based on the mechanism of its onset. First, we briefly review the findings
that PT-GVHD is induced by cytotoxic T-lymphocyte (CTL)-mediated
tissue injuries through the Fas/Fas ligand system, the
perforin/granzyme system, and alloantigen-specific antibodies
as well as through inflammatory cytokines. Secondly, we emphasize the
usefulness of a serine protease inhibitor for the inhibition of
CTL-mediated cytotoxicity in the earlier stages of onset. Subsequent
administration of methylprednisolone and 2-chlorodeoxyadenosine is
recommended for elimination of the donor's lymphocytes. The usefulness of
chloroquine for the suppression of CTL activity and the production of
tumor necrosis factor as well as the efficiency of pentoxyfylline for the
suppression of the production of tumor necrosis factor are also discussed
Therapeutic strategies for PT-GVHD should also be useful for
treating acute GVHD secondary to allogeneic bone marrow
transplantation, and to prevent the host's rejection of transplanted
organs as well as tissue damage in autoimmune diseases

L4 ANSWER 23 OF 37 MEDLINE   DUPLICATE 14
AN 1999321949 MEDLINE
DN 99321949 PubMed ID 10393698
TI Active participation of CCR5(+)CD8(+) T lymphocytes in the pathogenesis of
liver injury in graft-versus-host disease
AU Murai M, Yoneyama H, Harada A, Yi Z, Vestergaard C, Guo B, Suzuki K,
Asakura H, Matsushima K
CS Department of Molecular Preventive Medicine and CREST, School of
Medicine,
The University of Tokyo, Tokyo 113-0033, Japan
SO JOURNAL OF CLINICAL INVESTIGATION, (1999 Jul) 104 (1) 49-57
Journal code HS7, 7802877 ISSN 0021-9738
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199908
ED Entered STN 19990816
Last Updated on STN 19990816
Entered Medline 19990805
AB We examined the molecular pathogenesis of graft-versus
-host disease-associated (GVHD-associated) liver
injury in mice, focusing on the role of chemokines. At the second week
after cell transfer in the parent-into-F1 model of GVHD, CD8(+)
T cells -- especially donor-derived CD8(+) T cells -- infiltrated the
liver, causing both portal hepatitis and nonsuppurative destructive
cholangitis (NSDC) These migrating cells expressed CCR5 Moreover

macrophage inflammatory protein-1alpha (MIP-1alpha), one of the ligands
for CCR5, was selectively expressed on intralobular bile duct epithelial
cells, endothelial cells, and infiltrating macrophages and lymphocytes
Administration of anti-CCR5 antibody dramatically reduced the
infiltration of CCR5(+)CD8(+) T lymphocytes into the liver, and
consequently protected against liver damage in GVHD The levels
of Fas ligand (FasL) mRNA expression in the liver were
also decreased by anti-CCR5 antibody treatment. Anti-MIP-1alpha
antibody also reduced liver injury. These results
suggest that MIP-1alpha-induced migration of CCR5-expressing CD8(+) T
cells into the portal areas of the liver plays a significant role in
causing liver injury in GVHD thus, CCR5 and its ligand may be
the novel target molecules of therapeutic intervention of hepatic
GVHD

L4 ANSWER 24 OF 37 MEDLINE
AN 2000025609 MEDLINE
DN 20025609 PubMed ID 10555993
TI Effect of graft-versus-host disease (
GVHD) on host hematopoietic progenitor cells is mediated by Fas-
Fas ligand interactions but this does not explain the
effect of GVHD on donor cells.
AU Iwasaki T, Hamano T, Saheki K, Kuronwa T, Kataoka Y, Takemoto Y, Ogata
A,
CS Sugihara A, Terada N, Fujimoto J, Kakishita E
First Department of Internal Medicine, First Department of Pathology,
Second Department of Surgery, Hyogo College of Medicine, 1-1 Mukogawa-cho,
Nishinomiya, Hyogo, 663-8501, Japan
SO CELLULAR IMMUNOLOGY, (1999 Oct 10) 197 (1) 30-8
Journal code CQ9; 1246405 ISSN 0008-8749
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200001
ED Entered STN 20000114
Last Updated on STN 20000114
Entered Medline 20000104
AB The acute graft-versus-host disease (
GVHD) generated in BDF1 mice by the injection of spleen cells from
the C57BL/6 parental strain induces a direct cell-mediated attack on host
lymphohematopoietic populations, resulting in the reconstitution of the
host with donor hematopoietic stem cells. We examined the effect of
GVHD on the donor and host hematopoiesis in parental-induced acute
GVHD The bone marrow was hypoplastic and the number of
hematopoietic progenitor cells significantly decreased at 4 weeks after
GVHD induction. However, extramedullary splenic hematopoiesis was
present and the number of hematopoietic progenitor cells in the spleen
significantly increased at this time. Fas expression on the host spleen
cells and bone marrow cells significantly increased during weeks 2 to 8 of
GVHD. Host cell incubation with anti-Fas Ab induced apoptosis, and
the number of hematopoietic progenitor cells decreased during these weeks
A significant correlation between the augmented Fas expression on host
bone marrow cells and the decreased number of host bone marrow cells by
acute GVHD was observed. Furthermore, the injection of
Fas ligand (FasL)-deficient B6/gld spleen cells failed
to affect host bone marrow cells. Although Fas expression on repopulating
donor cells also increased. Fas-induced apoptosis by the repopulating
cells were donor cells. The number of hematopoietic progenitor cells in
the bone marrow and the spleen by the repopulating donor cells, however,
decreased over an extended time during acute GVHD. This suggests
that Fas-FasL interactions may regulate suppression of host hematopoietic
cells but not of donor hematopoietic cells. Hematopoietic dysfunctions
caused by the reconstituted donor cells are independent to Fas-FasL
interactions and persisted for a long time during parental-induced acute
GVHD
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L4 ANSWER 25 OF 37 MEDLINE   DUPLICATE 15
AN 1999261428 MEDLINE
DN 99261428 PubMed ID 9596649
TI Differential effects of anti-Fas ligand and anti-tumor
necrosis factor alpha antibodies on acute graft
versus-host disease pathologies

In addition to these immunosuppressive agents monoclonal antibodies against functional cell surface molecules, such as LFA-1, ICAM-1, Fas, and Fas ligand, must be effective for treatment of TA-GVHD Since the effective standard therapy of TA-GVHD has not been established the prevention by gamma irradiation of cellular blood components is most important

L4 ANSWER 31 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)
AN 97 877846 SCISEARCH
GA The Genuine Article (R) Number YG424
TI The synergistic effects of anti-Fas ligand and TNF-alpha antibody on the prevention of lethal acute graft-versus host disease in mice
AU Hattori K (Reprint), Hirano T, Tateno M, Oshimi K, Kayagaki N, Yagita H, Okumura K
CS JUNTENDO UNIV, SCH MED, SAPPORO CITY GEN HOSP, DEPT PATHOL, TOKYO 113, JAPAN, JUNTENDO UNIV, SCH MED, SAPPORO CITY GEN HOSP, DEPT INTERNAL MED, DIV HEMATOL, TOKYO 113 JAPAN, JUNTENDO UNIV SCH MED, SAPPORO CITY GEN HOSP, DEPT IMMUNOL, TOKYO 113 JAPAN
CYA JAPAN
SO BLOOD, (15 NOV 1997) Vol 90, No 10 Part 1, Supp (1), pp 907-907
Publisher W B SAUNDERS CO, INDEPENDENCE SQUARE WEST CURTIS CENTER, STE 300, PHILADELPHIA, PA 19106-3399
ISSN 0006-4971
DT Conference Journal
FS LIFE, CLIN
LA English
REC Reference Count 0

L4 ANSWER 32 OF 37 MEDLINE   DUPLICATE 17
AN 98062247 MEDLINE
DN 98062247 PubMed ID 9401075
TI Activity of TNF-related apoptosis-inducing ligand (TRAIL) in haematological malignancies
AU Snell V, Clodi K, Zhao S, Goodwin R, Thomas E K, Morris S W, Kadin M E, Cabanillas F, Andreeff M, Younes A
CS Department of Hematology, University of Texas M D Anderson Cancer Center, Houston 77030, USA
NC CA 01702 (NCI)
CA 21765 (NCI)
CA 69129 (NCI)
SO BRITISH JOURNAL OF HAEMATOLOGY (1997 Dec) 99 (3) 618-24
Journal code AXC, 0372544 ISSN 0007-1048
CY ENGLAND United Kingdom
DT Journal, Article, (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199801
Entered STN 19980206
Last Updated on STN 19980206
Entered Medline 19980206
Entered Medline 19980123
AB T-cell cytotoxicity is primarily mediated by two cell surface proteins, Fas ligand (FasL) and tumour necrosis factor-related apoptosis-inducing ligand (TRAIL), and intracellular perforin and granzyme granules. Fas-deficient and perforin-deficient T lymphocytes maintain cytotoxicity but fail to induce graft-versus-host disease (GVHD) when transplanted into mice suggesting that GVHD and graft-versus-tumour (GVT) effects can be dissociated, and that TRAIL is not involved in the pathogenesis of GVHD Because TRAIL could mediate a favourable GVT effect it became important to study the spectrum of its activity and to investigate factors that can dissociate its expression from FasL TRAIL induced apoptosis in 11/41 (27%) tumour specimens of haematological origin compared to 16/41 (39%) induced by FasL Although eight specimens were sensitive to both FasL and TRAIL no synergism was observed between these two ligands. TRAIL induced apoptosis in a dose and time dependent manner with an ED50 of 0.5 microg/ml and EDmax of 1 microg/ml TRAIL activity was not reduced by the over-expression of the multidrug resistant (MDR) protein, and was not enhanced by 9-cis retinoic acid (RA), which can down-regulate bcl-2 protein. Both ligands were simultaneously up-regulated in normal peripheral blood lymphocytes in response to IL-2, IL-15 and

anti-CD3 antibody whereas IL-10 had no effect Together, our data show that (1) TRAIL can mediate cell death in a variety of human haematological malignancies, (2) resistance to TRAIL is not mediated by MDR protein, (3) the lack of synergy between TRAIL and FasL suggests that either one is sufficient to mediate T-cell cytotoxicity, and (4) within the panel of cytokines tested, the expression of TRAIL and FasL could not be dissociated

L4 ANSWER 33 OF 37 MEDLINE   DUPLICATE 18
AN 97369712 MEDLINE
DN 97369712 PubMed ID 9226153
TI A metalloproteinase inhibitor prevents lethal acute graft-versus-host disease in mice
AU Hattori K, Hirano T, Ushiyama C, Miyajima H, Yamakawa N, Ebata T, Wada Y, Ikeda S, Yoshino K, Tateno M, Oshimi K, Kayagaki N, Yagita H, Okumura K
CS Department of Internal Medicine, Juntendo University School of Medicine, Bunkyo-Ku, Tokyo, Japan
SO BLOOD, (1997 Jul 15) 90 (2) 542-8
Journal code ABG, 7603509 ISSN 0006-4971
CY United States
DT Journal, Article, (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals, Priority Journals
EM 199708
ED Entered STN 19970813
Last Updated on STN 20000303
Entered Medline 19970807
AB Tumor necrosis factor (TNF) and Fas ligand (FasL) have been implicated in the pathogenesis of graft-versus-host disease (GVHD), which is a major complication after allogeneic bone marrow transplantation. We examined here the ameliorating effect of a metalloproteinase inhibitor (KB-R7785) that inhibits TNF-alpha and FasL release in a lethal acute GVHD model in mice. Administration of KB-R7785 into (BALBc x C57BL/6) F1 that received C57BL/6 spleen cells markedly reduced the mortality and weight loss in association with minimal signs of GVHD pathology in the liver, intestine, and hematopoietic tissues. The ameliorating effect of KB-R7785 was superior to that of anti-TNF-alpha antibody. Our results suggest that KB-R7785 could be a potent therapeutic agent for GVHD

L4 ANSWER 34 OF 37 CANCERLIT
AN 97619028 CANCERLIT
DN 97619028
TI Monocytes from cytokine mobilized stem cell products induce T cell apoptosis which is mediated in part by fas ligand (Meeting abstract)
AU Talmadge J E, Singh R K, Ageitos A, Ino K
CS University of Nebraska Medical Center, Omaha, NE
SO Proc Annu Meet Am Assoc Cancer Res, (1997) Vol 38, pp A235
ISSN 0197-016X
DT (MEETING ABSTRACTS)
FS ICDB
LA English
EM 199709
AB Cytokine mobilized peripheral blood stem cell (PSC) products have a high frequency of cells which inhibit T cell function. To determine the origin and mechanism of this activity, mobilized PSC products were fractionated by Percoll centrifugation and immunomagnetic bead. Monocytes, which constitute approximately forty percent of the cells within mobilized PSC products were found to inhibit T cell function via apoptosis. These cells inhibit both autologous and allogeneic T cell responses to PHA, pokeweed mitogen, OKT-3, IL-2, and tetanus toxin. The monocytes are low density, adherent and phagocytic, and are CD14+, HLA-DR+, CD11a+, CD1b+, CD56 (B7-2)+, CD80 (B7-1)-, CD16- and CD1a-. Cell-cell contact is required for inhibition and is not neutralized by antibodies against TNF-alpha However, the monocytes have increased mRNA levels for IL-10, and TNF compared to normal PBL. The inhibition of both autologous and allogeneic T cell proliferation was associated with apoptosis as measured by hypodiploidy and DNA fragmentation. Furthermore, some but not all of the inhibitory activity could be prevented with antibodies to CD95-ligand This immunoregulatory activity (via apoptosis) has potential to regulate immune recovery following myeloablative therapy and the removal of these cells from the PSC has therapeutic potential. Conversely, monocytes with T cell inhibitory activity have clinical potential for the control of allogeneic graft-versus-host disease or solid organ graft rejection

L4 ANSWER 35 OF 37 BIOSIS COPYRIGHT 2001 BIOSIS
AN 1998 67216 BIOSIS
DN PREV199800067216
TI The synergistic effects of anti-Fas ligand and TNF-alpha antibody on the prevention of lethal acute graft-versus-host disease in mice
AU Hattori, K (1), Hirano, T, Tateno, M, Oshimi, K, Kayagaki, N, Yagita, H, Okumura, K
CS (1) Div. Hematol., Dep. Internal Med., Juntendo Univ. Sch. Med., Tokyo Japan
SO Blood, (Nov. 15, 1997) Vol. 90, No. 10 SUPPL. 1 PART 1, pp. 206A
Meeting Info. 39th Annual Meeting of the American Society of Hematology San Diego, California, USA December 5-9, 1997 The American Society of Hematology
ISSN 0006-4971
DT Conference
LA English

L4 ANSWER 36 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)
AN 97 524588 SCISEARCH
GA The Genuine Article (R) Number XJ363
TI Fas-mediated cytotoxicity by intestinal intraepithelial lymphocytes during acute graft-versus-host disease in mice
AU Sakai T, Kimura Y, InagakiOhara K, Kusugami K, Lynch D H, Yoshikai Y (Reprint)
CS NAGOYA UNIV, SCH MED, DIS MECHANISM & CONTROL RES INST, LAB GERMFREE LIFE, SHOWA KU, 65 TSURUMAI CHO, NAGOYA, AICHI 468, JAPAN (Reprint), NAGOYA UNIV, SCH MED, DEPT INTERNAL MED 1, NAGOYA, AICHI 468, JAPAN, IMMUNEX CORP, DEPT IMMUNOL, SEATTLE, WA
CYA JAPAN, USA
SO GASTROENTEROLOGY, (JUL 1997) Vol 113, No 1, pp 168-174
Publisher W B SAUNDERS CO, INDEPENDENCE SQUARE WEST CURTIS CENTER, STE 300, PHILADELPHIA, PA 19106-5085
ISSN 0016-5085
DT Article, Journal
FS LIFE, CLIN
LA English
REC Reference Count 35
AB "ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS"
Background & Arms. Host-derived intestinal intraepithelial lymphocytes (IELs) increase in number during acute graft-versus-host disease (GVHD) in mice, in the present study, we examined Fas-mediated cytotoxicity by host-derived IELs against Fas-expressing target cells to see whether Fas/Fas ligand (Fas-L) interaction is involved in the pathogenesis of enteropathy during acute GVHD Methods. Acute GVHD was induced by injection of parental spleen cells into nonirradiated F1 mice The expression of Fas antigen on the intestinal epithelial cells (IECs) was examined by flow cytometry, and the expression of messenger RNA (mRNA) for Fas-L, interleukin 2, and interferon gamma in host-derived IELs was assessed by reverse-transcription polymerase chain reaction. Fas-mediated cytotoxicity by host-derived IELs was assessed using Fas-transfected cells, IECs, and Fas immunoglobulin Fc fusion protein (Fas Fc). Results. Fas antigen was constitutively expressed on the cell surface of IECs before and after GVHD induction Although Fas-L mRNA was not detected or detected scarcely in either alpha beta or gamma delta IELs before GVHD induction, both IELs expressed high levels of mRNA for Fas-L and interferon gamma after GVHD induction. Host-derived IELs during acute GVHD showed cytotoxicity against Fas-transfected target cells and IECs, which was partly blocked by addition of Fas Fc Conclusions. Fas/FasL-mediated cytotoxicity by host-derived IELs may be partly responsible for the enteropathy during acute GVHD

L4 ANSWER 37 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)
AN 95 42431 SCISEARCH
GA The Genuine Article (R) Number QA042

1) MASSIVE UP-REGULATION OF THE FAS LIGAND IN LPR AND GLD MICE - IMPLICATIONS FOR FAS REGULATION AND THE GRAFT-VERSUS-HOST DISEASE-LIKE WASTING SYNDROME
AU CHU J L., RAMOS P., ROSENDORFF A, NIKOLICZUGIC J, LACY E.
MATSUZAWA A. ELKON
K B (Reprint)
CS CORNELL UNIV, HOSP SPECIAL SURG MED CTR, SPECIALIZED CTR
RES SYST LUPUS
ERYTHEMATOSUS, NEW YORK, NY 10021 (Reprint), CORNELL UNIV.
HOSP SPECIAL SURG MED CTR, SPECIALIZED CTR RES SYST LUPUS
ERYTHEMATOSUS, NEW YORK, NY,
10021 MEM SLOAN KETTERING CANC CTR, PROGRAM MOLEC BIOL.
NEW YORK, NY,
10021 TOKAI UNIV, INST MED SCI, DEPT IMMUNOL, TOKYO 108, JAPAN,
TOKAI
UNIV INST MED SCI, DEPT INTERNAL MED, TOKYO 108, JAPAN
CYA USA JAPAN
SO JOURNAL OF EXPERIMENTAL MEDICINE, (01 JAN 1995) Vol 181, No 1,
PP 393-398
DT Note. Journal
    LIFE
    ISSN 0022-1007
    ENGLISH
C   Reference Count 29
AB *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*
Fas-deficient lpr and gld mice develop lymphadenopathy due to the
accumulation of T cells with an unusual double negative (DN)
(CD4(-)CD8(-)) phenotype. Previous studies have shown that these abnormal
cells are capable of inducing redirected lysis of certain Fc
receptor-positive target cells. Since the Fas ligand
(FasL) has recently been shown to be partly responsible for T
cell mediated cytotoxicity, lymph node cells from lpr and gld mice were
examined for the expression of FasL mRNA. Northern blot analysis revealed
that lymph node cells obtained from lpr and gld mice had a striking
increase in the level of expression of FasL mRNA predominantly due to
expression in the DN T cells. Furthermore, lpr, but not gld lymph node
cells killed the B cell line, A20, in a Fas-dependent manner. These
findings indicate that pas mutations result in a massive up-regulation of
FasL which, most likely, results from repetitive exposure to (self)
antigen. This phenomenon could explain the lpr-induced wasting syndrome
observed when lpr bone marrow-derived cells are acoptively transferred to
wild-type recipients.

=> file uspatfull

COST IN U S DOLLARS
                          SINCE FILE   TOTAL
                          ENTRY SESSION
FULL ESTIMATED COST   65 15   65 30

This file contains CAS Registry Numbers for easy and accurate
substance identification.

=> s 12 and (graft versus host or gvhd)

        1099 FAS
        30218 LIGAND
        112 FAS LIGAND
        (FAS(W)LIGAND)
        48222 ANTIBOD?
        25130 MONOCLON?
        31666 GRAFT
        112653 VERSUS
        96644 HOST
        1632 GRAFT VERSUS HOST
        (GRAFT(W)VERSUS(W)HOST)
        351 GVHD
   L5   17 L2 AND (GRAFT VERSUS HOST OR GVHD)

=> d l5 bib ab

L5   ANSWER 1 OF 17 USPATFULL
AN   2001 44200 USPATFULL
TI   Member of the TNF family useful for treatment and diagnosis of disease
IN   Wiley, Steven R., Libertyville, IL, United States
PA   Abbott Laboratories, Abbott Park, IL, United States (U S corporation)
PI   US 6207642 20010327
AI   US 1998-105543 19980626 (9)
RLI Continuation-in-part of Ser No US 1998-21706, filed on 10 Feb 1998,
     now abandoned Continuation-in-part of Ser No US 1997-798692, filed on
     12 Feb 1997, now abandoned
DT   Utility
EXNAM Primary Examiner, Romeo, David
LREP Becker, Cheryl L., Goller, Mimi C
CLMN Number of Claims, 2
ECL   Exemplary Claim: 1
DRWN 14 Drawing Figure(s), 9 Drawing Page(s)
LN CNT 4355
CAS INDEXING IS AVAILABLE FOR THIS PATENT
AB   An isolated clone consisting of sequences transcribed from the TREPA
     gene Also provided are human polypeptides translated from said TREPA
     sequences and a procedure for producing such polypeptide by recombinant
     techniques Also provided are a procedure for producing soluble
     biologically active TREPA, which may be used to treat deficiencies of
     TREPA and diseases conditions ameliorated by TREPA Antibodies,
     antagonists and inhibitors of such polypeptide which may be used to
     prevent the action of such polypeptide and therefore may be used
     therapeutically to treat TREPA-associated diseases, tumors or
     metastastases are disclosed Also disclosed is the use of said
     antibodies, agonists and inhibitors as well as the nucleic acid
     sequences to screen for, diagnose, prognosticate, stage and monitor
     conditions and diseases attributable to TREPA, especially inflammation
     The use of said partial sequence to provide antibodies,
     agonists and inhibitors as well as partial nucleic acid sequences to
     screen for, diagnose, stage and monitor diseases associated with TREPA,
     including but not limited to inflammation. Illustrative sequences and
     clone designations for TREPA are provided.

=> d l5 2-17 bib ab

L5   ANSWER 2 OF 17 USPATFULL
AN   2001 40270 USPATFULL
TI   Antisense inhibition of Fas mediated signaling
IN   Dean, Nicholas M., Olivenhain, CA, United States
     Marcusson, Eric G., San Diego, CA, United States
PA   Isis Pharmaceuticals, Inc., Carlsbad, CA, United States (U S
     corporation)
PI   US 6204055 20010320
AI   US 1999-290640 19990412 (9)
DT   Utility
EXNAM Primary Examiner Schwartzman, Robert A, Assistant Examiner
     Lacouciere, Karen A
LREP Law Offices of Jane Massey Licata
CLMN Number of Claims 39
ECL   Exemplary Claim 1
DRWN No Drawings
LN CNT 1930
CAS INDEXING IS AVAILABLE FOR THIS PATENT
AB   Compounds, compositions and methods are provided for inhibiting Fas
     mediated signaling The compositions comprise antisense compounds
     targeted to nucleic acids encoding Fas, FasL and Fap-1 Methods of using
     these antisense compounds for inhibition of Fas, FasL and Fap-1
     expression and for treatment of diseases, particularly autoimmune and
     inflammatory diseases and cancers, associated with overexpression or
     constitutive activation of Fas, FasL or Fap-1 are provided

L5   ANSWER 3 OF 17 USPATFULL
AN   2001 25915 USPATFULL
TI   Formamide compounds as therapeutic agents
IN   Andrews, Robert Carl, Durham, NC, United States
     Andersen, Marc Werner, Raleigh, NC, United States
     Bubacz, Dulce Garrido, Cary, NC, United States
     Chan, Joseph Howing, Chapel Hill, NC, United States
     Cowan, David John, Hillsborough, NC, United States
     Gaul, Michael David, Apex, NC, United States
     McDougald, Daryl Lynn, Durham, NC, United States
     Musso, David Lee, Raleigh, NC, United States
     Rabinowitz, Michael Howard, Durham, NC, United States
     Stanford, Jennifer Badiang, Cary, NC, United States
     Wiethe, Robert William, Durham, NC, United States
PA   Glaxo Wellcome Inc., Research Triangle Park, NC, United States (U S
     corporation)
PI   US 6191150 20010220
AI   US 1999-382747 19990825 (9)
PRAI GB 1998-18605   19980826
     US 1998-979959   19980826 (60)
DT   Utility
EXNAM Primary Examiner, Davis, Zinna Northington, Assistant Examiner
     Robinson, Binta
LREP Lemanowicz, John L.
CLMN Number of Claims 19
ECL   Exemplary Claim 1
DRWN No Drawings
LN CNT 2829
CAS INDEXING IS AVAILABLE FOR THIS PATENT
AB   A family of compounds having the general structural formula ##STR1##
     where W is a reverse hydroxamic acid group, and R sub 1, R sub 2,
     R sub 3, R sub 4, R sub 5 and R sub 6 are as described in the
     specification, or a pharmaceutically acceptable salt, solvate,
     biohydrolyzable ester, biohydrolyzable amide, affinity reagent, or
     prodrug thereof

L5   ANSWER 4 OF 17 USPATFULL
AN   2001 4738 USPATFULL
TI   Formamides as therapeutic agents
IN   Andrews, Robert Carl, Durham, NC, United States
     Andersen, Marc Werner, Raleigh, NC, United States
     Cowan, David John, Hillsborough, NC, United States
     Deaton, David Norman, Cary, NC, United States
     Dickerson, Scott Howard, Chapel Hill, NC, United States
     Drewry, David Harold, Durham, NC, United States
     Gaul, Michael David, Apex, NC, United States
     Luzzio, Michael Joseph, Durham, NC, United States
     Marion, Brian Edward, Durham, NC, United States
     Rabinowitz, Michael Howard, Durham, NC, United States (U S
PA   Glaxo Wellcome Inc., Research Triangle Park, NC, United States (U S
     corporation)
PI   US 6172064 20010109
AI   US 1999-362333 19990825 (9)
PRAI US 1998-97956   19980826 (60)
DT   Patent
EXNAM Primary Examiner Lambkin, Deborah C
LREP Lemanowicz, John L.
CLMN Number of Claims 21
ECL   Exemplary Claim 1
DRWN No Drawings

CAS INDEXING IS AVAILABLE FOR THIS PATENT
AB A family of compounds having the general structural formula ##STR1##

where W is a reverse hydroxamic acid group, and R sub 1, R sub 2, R sub 3, R sub 4, R sub 5 and R sub 6 are as described in the specification, or a pharmaceutically acceptable salt, solvate, biohydrolyzable ester, biohydrolyzable amide, affinity reagent, or prodrug thereof. Also described are methods for their preparation, pharmaceutical compositions including such compounds and their use in medicine

L5 ANSWER 5 OF 17 USPATFULL
AN 2000.167779 USPATFULL
TI Daxx, a Fas-binding protein that activates JNK and apoptosis
IN Yang, Xiaolu, Philadelphia, PA, United States
   Khosravi-Far, Roya, Malden, MA, United States
   Chang, Howard Y., Cambridge, MA, United States
PA Massachusetts Institute of Technology, Cambridge, MA, United States
   (U S corporation)
PI US 6159731 20001212
AI US 6159731 20001212
PRAI US 1998-22983 19980212 (9)
   US 1997-51753 19970626 (60)
DT Utility
EXNAM Primary Examiner: Elliott, George C.; Assistant Examiner: Shibuya, Mark
LREP Wolf, Greenfield & Sacks, P.C.
CLMN Number of Claims 14
ECL Exemplary Claim 1
DRWN 10 Drawing Figure(s), 9 Drawing Page(s)
LN CNT 2773
CAS INDEXING IS AVAILABLE FOR THIS PATENT
AB The invention describes nucleic acids encoding the Daxx protein, including fragments and biologically functional variants thereof. Also included are polypeptides and fragments thereof encoded by such nucleic acids, and antibodies relating thereto. Methods and products for using such nucleic acids and polypeptides also are provided

L5 ANSWER 6 OF 17 USPATFULL
AN 2000.160799 USPATFULL
TI Death domain containing receptors
IN Yu, Guo-Liang, Darnestown, MD, United States
   Ni, Jian, Rockville, MD, United States
   Gentz, Reiner L., Silver Spring, MD, United States
   Dillon, Patrick J., Gaithersburg, MD, United States
PA Human Genome Sciences, Inc., Rockville, MD United States (U S corporation)
PI US 6153402 20001128
AI US 6153402 20001128
PRAI US 1997-815469 19970311 (8)
   US 1996-13285 19960312 (60)
   US 1996-28711 19961017 (60)
   US 1997-37341 19970206 (60)
DT Utility
EXNAM Primary Examiner: Ulm, John
LREP Sterne, Kessler, Goldstein & Fox, P.L.L.C.
CLMN Number of Claims 61
ECL Exemplary Claim 1
DRWN 6 Drawing Figure(s), 10 Drawing Page(s)
LN CNT 3364
CAS INDEXING IS AVAILABLE FOR THIS PATENT
AB The present invention relates to novel Death Domain Containing Receptor (DR3 and DR3-V1) proteins which are members of the tumor necrosis factor (TNF) receptor family. In particular, isolated nucleic acid molecules are provided encoding the human DR3 and DR3-V1 proteins DR3 and DR3-V1 polypeptides are also provided, as are vectors, host cells and recombinant methods for producing the same. The invention further relates to screening methods for identifying agonists and antagonists of DR3 and DR3-V1 activity

L5 ANSWER 7 OF 17 USPATFULL
AN 2000.150164 USPATFULL
TI Sulfamide metalloprotease inhibitors
IN Broka, Chris Allen, Foster City, CA, United States
   Campbell, Jeffrey Allen, Fremont, CA, United States
   Castelhano, Arlindo Lucas, New City, NY, United States
   Chen, Jian Jeffrey, Santa Clara, CA, United States
   Hendricks, Robert Than, Palo Alto, CA, United States
   Melnick, Michael Joseph, San Diego, CA, United States
   Walker, Keith Adrian Murray, Los Altos Hills, CA, United States
PA Syntex (U S A) Inc., Palo Alto, CA, United States (U S corporation)
   Agouron Pharmaceuticals, Inc., San Diego, CA United States (U S corporation)
PI US 6143744 20001107
AI US 1999-369501 19990805 (9)
RLI Division of Ser No US 1998-9951, filed on 21 Jan 1998, now patented
PRAI US 1997-36714 19970123 (60)
   US 1997-62209 19971016 (60)
DT Utility
EXNAM Primary Examiner: Raymond, Richard L
LREP Peries, Rohan, Bansal, Rekha
CLMN Number of Claims 46
ECL Exemplary Claim 1
DRWN No Drawings
LN CNT 4788
CAS INDEXING IS AVAILABLE FOR THIS PATENT
AB This invention relates to sulfamides of formula (I) ##STR1## that are inhibitors of metalloproteases, pharmaceutical compositions containing them, methods for their use and methods for preparing these compounds

L5 ANSWER 8 OF 17 USPATFULL
AN 2000.134887 USPATFULL
TI Sulfamide metalloprotease inhibitors
IN Broka, Chris Allen, Foster City, CA, United States
   Campbell, Jeffrey Allen, Fremont, CA, United States
   Castelhano, Arlindo Lucas, New City, NY, United States
   Chen, Jian Jeffrey, Santa Clara, CA, United States
   Hendricks, Robert Than, Palo Alto, CA, United States
   Melnick, Michael Joseph, San Diego, CA, United States
   Walker, Keith Adrian Murray, Los Altos Hills, CA, United States
PA Syntex (USA) Inc., Palo Alto, CA, United States (U S corporation)
   Agouron Pharmaceuticals, Inc., San Diego, CA, United States (U S corporation)
PI US 6130220 20001010
AI US 1999-369677 19990805 (9)
RLI Division of Ser No US 1998-9951, filed on 21 Jan 1998
PRAI US 1998-36714 19980123 (60)
   US 1997-62209 19971016 (60)
DT Utility
EXNAM Primary Examiner: Shah, Mukund J.; Assistant Examiner: Schroeder, Ben
LREP Peries, Rohan, Bansal, Rekha
CLMN Number of Claims 44
ECL Exemplary Claim 1
DRWN No Drawings
LN CNT 5004
CAS INDEXING IS AVAILABLE FOR THIS PATENT
AB This invention relates to sulfamides of formula (I) ##STR1## that are inhibitors of metalloproteases, pharmaceutical compositions containing them, methods for their use and methods for preparing these compounds

L5 ANSWER 9 OF 17 USPATFULL
AN 2000.97998 USPATFULL
TI Agent for suppressing a reduction of CD4 sup + lymphocytes
IN Nakamura, Norio, Tokyo, Japan
   Shirakawa, Kamon, Tokyo, Japan
   Matsusue, Tomokazu, Tokyo, Japan
   Nagata, Shigekazu, Suita, Japan
   Co, Man Sung, Cupertino, CA, United States
   Vasquez, Maximiliano, Palo Alto, CA, United States
PA Mochida Pharmaceutical Co., Ltd., Tokyo, Japan (non-U S corporation)
   Osaka Bioscience Institute, Osaka-Fu, Japan (non-U S corporation)
PI US 6099631 20000801
AI US 1998-999631 19980122 (8)
RLI Continuation-in-part of Ser No US 1997-1011, filed on 30 Dec 1997, now abandoned which is a continuation-in-part of Ser No WO 1996-JP1820, filed on 1 Jul 1996 which is a continuation-in-part of Ser No US 1996-649100, filed on 17 May 1996
PRAI JP 1995-188480 19950630

DT Utility
EXNAM Primary Examiner: Kemmerer, Elizabeth; Assistant Examiner: Basi, Nirmal S
CLMN Number of Claims 4
ECL Exemplary Claim 1
DRWN No Drawings
LN CNT 615
CAS INDEXING IS AVAILABLE FOR THIS PATENT
AB An agent for preventing or treating AIDS which contains as its effective component an anti-Fas ligand antibody, and the method for preventing and treating AIDS by using such drug

L5 ANSWER 10 OF 17 USPATFULL
AN 2000.7385 USPATFULL
TI Soluble divalent and multivalent heterodimeric analogs of proteins
IN Schneck, Jonathan, Silver Spring, MD, United States
   O'Herin, Sean, Baltimore, MD, United States
PA The Johns Hopkins University, Baltimore, MD, United States (U S corporation)
PI US 6015884 20000118
AI US 1997-828712 19970328 (8)
PRAI US 1996-14367 19960328 (60)
DT Utility
EXNAM Primary Examiner: Hutzell, Paula K.; Assistant Examiner: Bansal, Geetha P
LREP Banner & Witcoff, Ltd
CLMN Number of Claims 10
ECL Exemplary Claim 1
DRWN 18 Drawing Figure(s), 16 Drawing Page(s)
LN CNT 2027
CAS INDEXING IS AVAILABLE FOR THIS PATENT
AB Specificity in immune responses is in part controlled by the selective interaction of T cell receptors with their cognate ligands, peptide/MHC molecules. The discriminating nature of this interaction makes these molecules, in soluble form, good candidates for selectively regulating immune responses. Attempts to exploit soluble analogs of these proteins has been hampered by the intrinsic low avidity of these molecules for their ligands. To increase the avidity of soluble analogs for their cognates to biologically relevant levels, divalent peptide/MHC complexes or T cell receptors (superdimers) were constructed. Using a recombinant DNA strategy, DNA encoding either the MHC class II peptide or TCR heterodimers was ligated to DNA coding for murine Ig heavy and light chains. These constructs were subsequently expressed in a baculovirus expression system. Enzyme-linked immunosorbent assays (ELISA) specific for the Ig and polymorphic determinants of either the TCR or MHC fraction of the molecule indicated that infected insect cells secreted approximately 1 mg/ml of soluble, conformationally intact chimeric superdimers. SDS PAGE gel analysis of purified protein showed that expected molecular weight species. The results of flow cytometry demonstrated that the TCR and class II chimeras bound specifically with high avidity to cells bearing their cognate receptors. These superdimers will be useful for studying TCR/MHC interactions, lymphocyte tracking, identifying new antigens, and have possible uses as specific regulators of immune responses

L5 ANSWER 11 OF 17 USPATFULL
AN 2000.7061 USPATFULL
TI Fas antagonists
IN Lynch, David H., Bainbridge Island, WA, United States
   Alderson, Mark R., Bainbridge Island, WA, United States
PA Immunex Corporation, Seattle, WA, United States (U S corporation)
PI US 6015569 20000118
AI US 1998-152733 19980914 (9)
RLI Division of Ser No US 1995-429499, filed on 26 Apr 1995, now patented Pat No US 5830469 which is a continuation-in-part of Ser No US 1994-322805, filed on 13 Oct 1994, now patented. Pat No US 5620289 which is a continuation-in-part of Ser No US 1993-159003, filed on 29 Nov 1993, now abandoned which is a continuation-in-part of Ser No US 1993-136817, filed on 14 Oct 1993, now abandoned
DT Utility
EXNAM Primary Examiner: Chin, Christopher L.; Assistant Examiner: Dev, S
LREP Anderson, Kathryn A
CLMN Number of Claims 17
ECL Exemplary Claim 1
DRWN 14 Drawing Figure(s), 10 Drawing Page(s)

.N CNT 2134
CAS INDEXING IS AVAILABLE FOR THIS PATENT
AB   The present invention provides a panel of monoclonal antibodies and binding proteins which specifically bind to human Fas antigen Some of the antibodies and binding proteins are capable of stimulating T cell proliferation, inhibiting binding of anti-Fas CH-11 monoclonal antibody to cells expressing Fas antigen, blocking anti-Fas CH-11 monoclonal antibody-mediated lysis of cells The invention also provides for therapeutic compositions comprising the monoclonal antibodies

L5   ANSWER 12 OF 17 USPATFULL
AN   1999 160028 USPATFULL
TI   Sulfamide-metalloprotease inhibitors
IN   Broka, Chris Allen, Foster City, CA, United States
      Campbell, Jeffrey Allen, Fremont, CA, United States
      Castelhano, Arlindo Lucas, New City, NY, United States
      Chen, Jian Jeffrey, Santa Clara, CA, United States
      Hendricks, Robert Than, Palo Alto, CA, United States
      Melnick, Michael Joseph, San Diego, CA, United States
      Walker, Keith Adrian Murray, Los Altos Hills, CA, United States
PA   Syntex (U S A) Inc., Palo Alto, CA, United States (U S corporation)
      Agouron Pharmaceuticals, Inc., San Diego, CA, United States (U S corporation)
PI   US 5998412 19991207
AI   US 1998-9951 19980121 (9)
PRAI US 1997-36714 19970123 (60)
      US 1997-62209 19971016 (60)
DT   Utility
EXNAM Primary Examiner Raymond, Richard L.
LREP Penies, Rohan, Bansal, Rekha
CLMN Number of Claims 15
ECL   Exemplary Claim 1
DRWN No Drawings
CAS INDEXING IS AVAILABLE FOR THIS PATENT
AB   This invention relates to sulfamides of formula (I) ##STR1## that are inhibitors of metalloproteases, pharmaceutical compositions containing them, methods for their use and methods for preparing these compounds

L5   ANSWER 13 OF 17 USPATFULL
AN   1999 89171 USPATFULL
TI   Matrix metalloprotease inhibitors
IN   Bender, Steven Lee, Oceanside, CA, United States
      Broka, Chris Allen, Foster City, CA, United States
      Campbell, Jeffrey Allen, Fremont, CA, United States
      Castelhano, Arlindo Lucas, New York, NY, United States
      Fisher, Lawrence Emerson, Mountain View, CA, United States
      Hendricks, Robert Than, Palo Alto, CA, United States
      Sarma, Keshab, Sunnyvale, CA, United States (U S corporation)
PA   Syntex (U S A) Inc., Palo Alto, CA, United States (U S corporation)
      Agouron Pharmaceuticals, Inc., San Diego, CA, United States (U S corporation)
PI   US 5932595 19990803
AI   US 1996-769049 19961218 (8)
PRAI US 1996-22439 19960807 (60)
      US 1995-8939 1995 1220 (60)
      US 1996-32096 1996 1204 (60)
DT   Utility
EXNAM Primary Examiner Kight, John, Assistant Examiner Covington, Raymond
LREP Penies, Rohan
CLMN Number of Claims 60
ECL   Exemplary Claim 1
DRWN No Drawings
LN CNT 4966
CAS INDEXING IS AVAILABLE FOR THIS PATENT
AB   The present invention relates to compounds of Formula I, ##STR1## that are matrix metalloprotease inhibitors, pharmaceutical compositions containing them, methods for their use and methods of preparing these compounds

L5   ANSWER 14 OF 17 USPATFULL
AN   1999 4647 USPATFULL

TI   Fas ligand compositions for treatment of proliferative disorders
IN   Walsh, Kenneth, Carlisle, MA, United States
PA   St. Elizabeth's Medical Center, Boston, MA, United States (U S corporation)
PI   US 5858990 19990112
AI   US 1997-810453 19970304 (8)
DT   Utility
EXNAM Primary Examiner Elliott, George C., Assistant Examiner McGarry, Sean
LREP Wolf, Greenfield & Sacks, P C.
CLMN Number of Claims 9
ECL   Exemplary Claim 1
DRWN No Drawings
LN CNT 3038
CAS INDEXING IS AVAILABLE FOR THIS PATENT
AB   A method for treating vascular injury, particularly vascular injury resulting from restenosis following angioplasty, and vascular remodeling is provided. The method involves administering to subjects in need of such treatment an effective amount of a Fas ligand molecule

L5   ANSWER 15 OF 17 USPATFULL
AN   1998 134631 USPATFULL
TI   Fas antagonists and uses thereof
IN   Lynch, David H., Bainbridge Island WA, United States
      Alderson, Mark R., Bainbridge Island, WA, United States
PA   Immunex Corporation, Seattle, WA, United States (U S corporation)
PI   US 5830469 19981103
AI   US 1995-429499 19950426 (8)
RLI   Continuation-in-part of Ser. No US 1994-322805, filed on 13 Oct 1994, now patented, Pat No. US 5620889 which is a continuation-in-part of Ser. No. US 1993-159003, filed on 29 Nov 1993, now abandoned which is a continuation-in-part of Ser No. US 1993-136817, filed on 14 Oct 1993, now abandoned
DT   Utility
EXNAM Primary Examiner Loring, Susan A.
LREP Anderson, Kathryn A.
CLMN Number of Claims 28
ECL   Exemplary Claim 1
DRWN 14 Drawing Figure(s), 10 Drawing Page(s)
LN CNT 1997
CAS INDEXING IS AVAILABLE FOR THIS PATENT
AB   The present invention provides a panel of monoclonal antibodies and binding proteins which specifically bind to human Fas antigen Some of the antibodies and binding proteins are capable of stimulating T cell proliferation, inhibiting binding of anti-Fas CH-11 monoclonal antibody to cells expressing Fas antigen, blocking anti-Fas CH-11 monoclonal antibody-mediated lysis of cells, and blocking Fas ligand-mediated lysis of cells The invention also provides for therapeutic compositions comprising the monoclonal antibodies

L5   ANSWER 16 OF 17 USPATFULL
AN   1998 134627 USPATFULL
TI   Yeast-based delivery vehicles
IN   Duke, Richard C., Denver, CO, United States
      Franzusoff, Alex, Boulder, CO, United States
      Bellgrau, Donald, Denver, CO, United States
PA   University Technology Corporation, Boulder, CO, United States (U S corporation)
PI   US 5830463 19981103
AI   US 1994-340185 19941115 (8)
RLI   Continuation-in-part of Ser. No US 1993-88322, filed on 7 Jul 1993, now patented, Pat No US 5413914
DT   Utility
EXNAM Primary Examiner Chambers, Jasemine C., Assistant Examiner Hauda, Karen M
LREP Sheridan Ross P C,
CLMN Number of Claims 12
ECL   Exemplary Claim 1, 12
DRWN 3 Drawing Figure(s), 3 Drawing Page(s)
LN CNT 1929
CAS INDEXING IS AVAILABLE FOR THIS PATENT

AB   The present invention includes yeast vehicles and their use as delivery vehicles. Yeast vehicles include a yeast portion and a heterologous compound Such yeast vehicles can be used to protect animals from disease and to otherwise carry compounds to given cell types Examples of yeast vehicles include gene delivery vehicles, drug delivery vehicles, and immunomodulatory vehicles Immunomodulatory vehicles are capable of modulating an immune response When stimulating an immune response, such yeast vehicles effect cell-mediated as well as humoral immunity

L5   ANSWER 17 OF 17 USPATFULL
AN   1998 61156 USPATFULL
TI   Use of fas ligand to supress T-lymphocyte-mediated immune responses
IN   Bellgrau, Donald, Denver, CO, United States
PA   Duke, Richard C., Denver, CO, United States
      University Technology Corporation, Boulder, CO, United States (U S corporation)
PI   US 5759536 19980602
AI   US 1995-378507 19950126 (8)
RLI   Continuation-in-part of Ser No. US 1994-250478, filed on 27 May 1994, now abandoned
DT   Utility
EXNAM Primary Examiner Campbell, Bruce R
LREP Sheridan & Ross, P C
CLMN Number of Claims 7
ECL   Exemplary Claim 1
DRWN No Drawings
LN CNT 802
CAS INDEXING IS AVAILABLE FOR THIS PATENT
AB   A method for inhibiting T-lymphocyte-mediated immune responses, including those directed against autologous and/or heterologous tissues, e.g., by a recipient mammal of a transplanted tissue, said method comprising providing the recipient mammal with Fas ligand. The Fas ligand may be provided to the recipient mammal by a variety of means, including by pump implantation or by transplantation of transgenic tissue expressing Fas ligand. Also provided is a method for diagnostic use of Fas ligand expression in improving transplantation success

=> d his

=> logoff

(FILE 'HOME' ENTERED AT 10:02:45 ON 25 MAY 2001)

FILE 'MEDLINE, BIOSIS, EMBASE, CANCERLIT, SCISEARCH' ENTERED
AT 10:03:22 ON 25 MAY 2001
L1   11361 S FAS LIGAND
L2   3148 S L1 AND (ANTIBOD? OR MONOCLON?)
L3   88 S L2 AND (GRAFT VERSUS HOST OR GVHD)
L4   37 DUP REM L3 (51 DUPLICATES REMOVED)

FILE 'USPATFULL' ENTERED AT 10:13:02 ON 25 MAY 2001
L5   17 S L2 AND (GRAFT VERSUS HOST OR GVHD)

=> logoff

ALL L# QUERIES AND ANSWER SETS ARE DELETED AT LOGOFF
LOGOFF? (Y)/N/HOLD y

COST IN U S DOLLARS   SINCE FILE   TOTAL
                                   ENTRY   SESSION
FULL ESTIMATED COST   29 06   94 36

STN INTERNATIONAL LOGOFF AT 10 14 06 ON 25 MAY 2001

AU Hattori K Hirano T Miyajima H Yamakawa N Tateno M Oshimi K
Kayagaki N Yagita H Okumura K
CS Division of Hematology, Department of Internal Medicine, Juntendo
University School of Medicine. Tokyo, Japan
SO BLOOD, (1998 Jun 1) 91 (11) 4051-5.
Journal code A8G, 7603509 ISSN 0006-4971.
CY United States
DT Journal Article (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals, Priority Journals
EM 199806
ED Entered STN 19980713
Last Updated on STN 19980713
Entered Medline 19980626
AB Both tumor necrosis factor alpha (TNFalpha) and Fas
ligand (FasL) have been implicated in the pathogenesis of
graft-versus-host disease (GVHD). In
this study, we examined the ameliorating effects of neutralizing anti-FasL
and/or anti-TNFalpha monoclonal antibody (MoAb) in a
lethal acute GVHD model in mice. Whereas the treatment with
either anti-FasL or anti-TNFalpha MoAb alone significantly delayed the
mortality and improved the body weight, a complete protection was achieved
by the administration of both MoAbs. Pathological examination indicated
differential effects of anti-FasL or anti-TNFalpha MoAb on GVHD
-associated pathologies. Hepatic lesion was improved by anti-FasL but not
anti-TNFalpha MoAb In contrast, intestinal lesion was improved by
anti-TNFalpha but not anti-FasL MoAb The combination of both MoAbs improved all these
lesions. These results indicate that FasL and TNFalpha differentially
contribute to the GVHD pathologies and a complete protection
from mortality can be achieved by neutralization of both FasL and
TNFalpha

L4 ANSWER 26 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)
AN 1998 706176 SCISEARCH
GA The Genuine Article (R) Number: 118WU
TI Differential expression of Fas and Fas ligand in acute
and chronic graft-versus-host disease
Up-regulation of Fas and Fas ligand requires CD8(+) T
cell activation and IFN-gamma production
AU Shustov A, Nguyen P, Finkelman F, Elkon K B, Via C S (Reprint)
CS UNIV MARYLAND, SCH MED, DIV CLIN IMMUNOL & RHEUMATOL,
MSTF 8-34, 10 S PINE
ST, BALTIMORE, MD 21201 (Reprint), UNIV MARYLAND, SCH MED, DIV
CLIN
IMMUNOL & RHEUMATOL, BALTIMORE, MD 21201, DEPT VET AFFAIRS
MED CTR, RES
SERV, BALTIMORE, MD, VET AFFAIRS MED CTR, DIV RHEUMATOL,
CINCINNATI OH
45267, UNIV CINCINNATI, COLL MED, CINCINNATI, OH 45267, CORNELL
UNIV, MED
CTR, HOSP SPECIAL SURG, SPECIALIZED CTR RES SYSTEM LUPUS
ERYTHEMATOSUS,
NEW YORK, NY 10021
CYA USA
SO JOURNAL OF IMMUNOLOGY, (15 SEP 1998) Vol 161 No 6, pp 2848-
2855
Publisher AMER ASSOC IMMUNOLOGISTS, 9650 ROCKVILLE PIKE,
BETHESDA, MD
20814
ISSN 0022-1767
DT Article, Journal
FS LIFE
LA English
REC Reference Count 37
AB *ABSTRACT IS AVAILABLE IN THE ALL AND (ALL FORMATS)*
The parent-into-F-1 model of acute and chronic graft-vs-host disease (
GVHD) was used as an example of in vivo cell-mediated or
Ab-mediated responses, respectively, and the roles of Fas and Fas
ligand (FasL) were investigated. Using both how cytometry and PCR
methodologies, we found that acute GVHD mice exhibited
significant up-regulation of Fas and FasL, whereas Fas/FasL up-regulation
in chronic GVHD mice was equal to or marginally greater than
that in uninjected mice. Functional studies confirmed that Fas/FasL
contributed to the anti-host CTL activity of splenocytes from acute

GVHD mice, although a perforin-dependent pathway was also
identified Despite the presence of Fast on both donor CD4(+) and CD8(+) T
cells in acute GVHD mice, depletion studies demonstrated that
all the in vitro anti-host CTL activity resided in the CD8(+) population.
Furthermore, injection of CD8-depleted B6 spleen cells into F-1 mice
blocked Fas/FasL up-regulation and IFN-gamma production, resulting in
chronic GVHD. Lastly, up-regulation of Fas/FasL in acute
GVHD mice could be blocked by anti-IFN-gamma mAb in vivo Thus, in
this in vivo model of alloantigen immune responsiveness, Fas/FasL
up-regulation is critically dependent on Ag-specific (donor) CD8(+) T cell
activation and IFN-gamma production. Donor CD4(+) T cell activation in the
absence of CD8(+) T cell activation results in an autoantibody-mediated
response, no significant Fas/FasL up-regulation, impaired elimination of
autoreactive B cells, and persistent humoral autoimmunity

L4 ANSWER 27 OF 37 EMBASE COPYRIGHT 2001 ELSEVIER SCI B V
AN 1998337925 EMBASE
TI GVHD after small bowel transplantation: The role of caspases,
FASL, and galectin-1
AU Fandrich F; Zepernick-Kalinski C; Lin X
CS Dr F Fandrich, Dept of General/Thoracic Surgery, University of Kiel,
Arnold-Heller-Str 7, 24105 Kiel, Germany
SO Transplantation Proceedings, (1998) 30/6 (2594-2595)
Refs: 6
ISSN 0041-1345 CODEN TRPPA8
PUI S 0041-1345(98)00743-X
CY United States
DT Journal; Conference Article
FS 005 General Pathology and Pathological Anatomy
009 Surgery
026 Immunology, Serology and Transplantation
037 Drug Literature Index
LA English

L4 ANSWER 28 OF 37 MEDLINE   DUPLICATE 16
AN 1998182300 MEDLINE
DN 98182300 PubMed ID 9516135
TI Induction of Fas (Apo-1, CD95)-mediated apoptosis of activated lymphocytes
by polyclonal antithymocyte globulins.
AU Genestier L, Fournel S, Flacher M, Assossou O, Revillard J P,
Bonnefoy-Berard N
CS Laboratory of Immunology, INSERM, Hopital E Herriot, Lyon, France
SO BLOOD, (1998 Apr 1) 91 (7) 2360-8.
Journal code A8G, 7603509 ISSN 0006-4971.
CY United States
DT Journal, Article, (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals, Priority Journals
EM 199804
ED Entered STN 19980422
Last Updated on STN 19980422
Entered Medline 19980415
AB Polyclonal horse antilymphocyte and rabbit antithymocyte globulins (ATGs)
are currently used in severe aplastic anemia and for the treatment of
organ allograft acute rejection and graft-versus-
host disease. ATG treatment induces a major depletion of
peripheral blood lymphocytes, which contributes to its overall
immunosuppressive effects. Several mechanisms that may account for
lymphocyte lysis were investigated in vitro. At high concentrations (1 to
1 mg/mL) ATGs activate the human classic complement pathway and induce
lysis of both resting and phytohemagglutinin (PHA) activated peripheral
blood mononuclear cells. At low, submitogenic, concentration ATGs induce
antibody-dependent cell cytotoxicity of PHA-activated cells, but
not resting cells. They also trigger surface Fas (Apo-1, CD95) expression
in naive T cells and Fas-ligand gene and protein
expression in both naive and primed T cells, resulting in Fas/Fas-L
interaction-mediated cell death. ATG-induced apoptosis and Fas-L
expression were not observed with an ATG preparation lacking CD2 and CD3
antibodies. Susceptibility to ATG-induced apoptosis was restricted
to activated cells, dependent on IL-2, and prevented by Cyclosporin A,
FK506, and rapamycin. The data suggest that low doses of ATGs could be
clinically evaluated in treatments aiming at the selective deletion of in
vivo activated T cells in order to avoid massive lymphocyte depletion and
subsequent immunodeficiency

L4 ANSWER 29 OF 37 SCISEARCH COPYRIGHT 2001 ISI (R)

AN 1998 839528 SCISEARCH
GA The Genuine Article (R) Number: 133NJ
TI Increased soluble Fas-ligand in sera of bone marrow
transplant recipients with acute graft-versus-
host disease
AU Kanda Y, Tanaka Y, Yatomi T, Nakamura N, Kami M, Saito T,
Izutsu K, Asai T, Yuji K, Ogawa S, Honda H, Mitani K, Chiba S, Yazaki Y,
Hirai H (Reprint)
CS UNIV TOKYO, FAC MED, DEPT CELL THERAPY & TRANSPLANT AT
MED, BUNKYO KU,
7-3-1 HONGO, TOKYO 113, JAPAN (Reprint), UNIV TOKYO, FAC MED
DEPT CELL
THERAPY & TRANSPLANT AT MED, BUNKYO KU, TOKYO 113, JAPAN,
UNIV TOKYO, FAC
MED, DEPT INTERNAL MED 3, TOKYO 113, JAPAN, MOCHIDA
PHARMACEUT CO, BIOSCI
RES LAB, TOKYO, JAPAN
CYA JAPAN
SO BONE MARROW TRANSPLANTATION, (OCT 1998) Vol 22, No 8, pp
751-754
Publisher STOCKTON PRESS, HOUNDMILLS, BASINGSTOKE RG21 6XS,
HAMPSHIRE,
ENGLAND
ISSN 0268-3369
DT Article, Journal
FS LIFE, CLIN
LA English
REC Reference Count 19
AB *ABSTRACT IS AVAILABLE IN THE ALL AND (ALL FORMATS)*
Acute graft-versus-host disease (aGVHD)
is a major complication following allogeneic bone marrow transplantation
(BMT). Recently, accumulating evidence indicates that the Fas/FasFas
ligand (FasL) system is implicated on in the pathogenesis of aGVHD in
murine models. We determined the serum levels of soluble Fast (sFasL) in
BMT recipients using an enzyme-linked immunosorbent assay The serum
sFasL
was suppressed during the period of myelosuppression following the
preparative regimen and subsequently increased with hematopoietic
reconstitution after BMT. In patients with aGVHD, the serum sFasL level
was significantly higher than in those without aGVHD. In the mixed
lymphocyte reaction assay, sFasL in the supernatants was increased with a
significant correlation to the level of H-3-thymidine uptake. Our findings
suggest that the Fas/FasL system is activated by allogeneic stimulation
and may have close correlation to the development of aGVHD in human
BMT

L4 ANSWER 30 OF 37 MEDLINE
AN 97466817 MEDLINE
DN 97466817 PubMed ID 9301292
TI Treatment of transfusion-associated graft-versus-
host disease
AU Yasukawa M
CS First Department of Internal Medicine, Ehime University School of
Medicine
SO NIPPON RINSHO. JAPANESE JOURNAL OF CLINICAL MEDICINE, (1997
Sep) 55 (9)
2290-5. Ref 13
Journal code: KIM, 0420546 ISSN 0047-1852
CY Japan
DT Journal, Article, (JOURNAL ARTICLE)
General Review, (REVIEW)
(REVIEW LITERATURE)
LA Japanese
FS Priority Journals
EM 199712
ED Entered STN 19980109
Last Updated on STN 19980109
Entered Medline 19971216
AB Transfusion-associated graft-versus-host
disease (TA-GVHD) in immunocompetent patients is mediated by
activated lymphocytes derived from the donor directed against host
allogeneic HLA antigens. When considering this pathogenesis, the target of
TA -GVHD treatment should be focused on the cytotoxic T cells
(CTL) directed against host HLA The combination therapy of anti-CD3
monoclonal antibody, OKT3, cyclosporin A, and
corticosteroid may be effective to inhibit the cytotoxic activity of CTL

Induction of Fas (Apo-1, CD95)-Mediated Apoptosis of Activated Lymphocytes by Polyclonal Antithymocyte Globulins

By Laurent Genestier, Sylvie Fournel, Monique Flacher, Olga Assossou, Jean-Pierre Revillard, and Nathalie Bonnefoy-Berard

Polyclonal horse antilymphocyte and rabbit antithymocyte globulins (ATGs) are currently used in severe aplastic anemia and for the treatment of organ allograft acute rejection and graft-versus-host disease. ATG treatment induces a major depletion of peripheral blood lymphocytes, which contributes to its overall immunosuppressive effects. Several mechanisms that may account for lymphocyte lysis were investigated in vitro. At high concentrations (.1 to 1 mg/mL) ATGs activate the human classic complement pathway and induce lysis of both resting and phytohemagglutinin (PHA)-activated peripheral blood mononuclear cells. At low, submitogenic, concentration ATGs induce antibody-dependent cell cytotoxicity of PHA-activated cells, but not resting cells. They also trigger surface Fas (Apo-1, CD95) expression in naive T cells and Fas-ligand gene and protein expression in both naive and primed T cells, resulting in Fas/Fas-L interaction-mediated cell death. ATG-induced apoptosis and Fas-L expression were not observed with an ATG preparation lacking CD2 and CD3 antibodies. Susceptibility to ATG-induced apoptosis was restricted to activated cells, dependent on IL-2, and prevented by Cyclosporin A, FK506, and rapamycin. The data suggest that low doses of ATGs could be clinically evaluated in treatments aiming at the selective deletion of in vivo activated T cells in order to avoid massive lymphocyte depletion and subsequent immunodeficiency.
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THE POLYCLONAL antilymphocyte or antithymocyte globulins (ATG)* are potent immunosuppressive agents used in organ transplantation since the late 1960s. They have proved effective either as rescue treatment of first rejection episodes and graft-versus-host reaction or as prophylactic treatment of rejection.[1] As an alternative to polyclonal ATGs, monoclonal antibody (MoAb) OKT3 has been extensively used in organ transplantation.[2,3] However, in clinical studies, polyclonal ATGs compare favorably to OKT3 both for prophylactic use or in rescue therapy.[4] The precise mechanism of action of ATGs is undefined, but the profound lymphocytopenia observed throughout the treatment period mainly contributes to the immunosuppressive effect. Various mechanisms have been proposed to explain lymphocyte depletion, including complement-mediated cytolysis or clearance of lymphocytes by opsonization and phagocytosis by macrophages.[5] ATGs are a mixture of multiple antibodies to various lymphocyte surface antigens.[6-8] It was recently reported that antibodies specific for HLA class I molecules,[9-11] and antibodies to CD2,[12,13] CD30,[14] CD45,[15] and CTLA-4[16] could induce apoptosis of T cells, whereas anti-HLA class II and anti-HLA class I antibodies can also trigger apoptosis of activated B cells.[17] Antibodies to CD2, CD3, CD45, and HLA molecules were identified in ATGs; it may therefore be hypothesized that their binding either to

resting or to activated T cells, or both, may trigger a signal of programmed cell death. Furthermore, ATGs contain antibodies to CD2 and CD3, which account for their mitogenic properties.[7] Repeated activation of mature T cells through CD2 or CD3 results in apoptosis of activated T cells.[18] The major pathway of this activation-induced cell death (AICD) uses the interaction between Fas (Apo-1, CD95) expressed by activated T and B cells and Fas-ligand (Fas-L, CD95-L) produced by a subset of activated T cells.[19-21] The present study was designed to investigate in vitro the different mechanisms whereby ATGs can induce peripheral lymphocyte depletion. To this end, we measured the capacity of ATGs bound to peripheral blood lymphocytes (PBL) to bind human C1q and to induce complement-dependent lysis. We determined their activity in antibody-dependent cell-mediated cytotoxicity (ADCC) and their capacity to induce Fas and Fas-L expression. In all those assays, we compared the sensitivity of naive versus mitogen-activated PBL to ATG-induced lysis, in order to identify those mechanisms that could display some specificity toward preactivated PBL. The dose responses were analyzed according to serum concentrations achieved during treatments. Finally, we evaluated the effect of immunosuppressive drugs that interfere with the interleukin-2 (IL-2) pathway (Cyclosporin A, [CsA], FK506, rapamycin) on the development of the sensitivity to ATG-induced lysis.

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*Within the context of this report, ATG is used to refer to either antithymocyte or antilymphocyte globulins.

MATERIALS AND METHODS

Antibodies and reagents. Rabbit ATG, batch no. 95-07, and horse antilymphocyte globulins, batches no. 1141 and no. 5, were provided by Dr J. Carcagne (Pasteur Merieux serums & vaccins, Lyon, France). Characteristics of each batch have been previously reported.[7] F(ab')₂ fragments of ATG no. 95-07 were prepared by pepsin digestion and purified by exclusion chromatography on protein A, following standard procedures. Normal rabbit IgG (Zymed, San Francisco, CA) and horse anti-rabies globulins purified according to the same procedure used for ATGs (Pasteur Merieux serums & vaccins) were used as controls. The anti-CD52 MoAb CAMPATH-1M (IgM) was a gift from Prof H. Waldmann (Sir Dunn School of Pathology, University of Oxford, Oxford, UK). The three anti-Fas MoAbs were used in this study, UB2 for cytofluorometry assays; CH11 (IgM), ZB4 (IgG1), and phycoerythrine streptavidin were obtained from Immunotech (Marseille, France).

Fluorescein-isothiocyanate (FITC)-conjugated CD25 and CD69 MoAbs were obtained from Becton Dickinson (Mountain View, CA) and two biotinylated anti-Fas-L, one from Pharmingen (San Diego, CA) and the other from Alexis Corporation (Coger S.A., Paris, France). CD3 MoAb OKT3 was from Cilag Laboratories (Levallois-Perret, France).

The lectin phytohemagglutinin (PHA), phorbol myristate acetate (PMA), ionomycin, and cycloheximide (CHX) were obtained from Sigma Chemical Co. (St Louis, MO). Rapamycin (RPM) and FK506 were gifts from Dr A. Altmann (La Jolla Institute for Allergy and Immunology, San Diego, CA), and CsA was kindly supplied by Sandoz (Novartis, Paris, France). Human IL-2 and rIFN-γ were kindly provided by Dr J. Banchereau (Schering-Plough, Dardilly, France).

Cell preparation. Peripheral blood was collected from healthy donors in the presence of sodium citrate. After the addition of a calcium chloride solution, blood was defibrinated by gentle rotation of the flask; mononuclear cells were then isolated by centrifugation on a layer of Histopaque (Sigma). Cells were washed three times in Hank's balanced salt solution (HBSS) before culture. Those cell suspensions referred to as PBL were shown to contain 3.8% + 0.4% monocytes, as defined by expression of CD14. For complement-mediated lysis and ADCC experiments, peripheral blood mononuclear cells (PBMC) were obtained by centrifugation of heparinized blood on a layer of Histopaque.

Culture medium and cell proliferation. PBL were resuspended in RPMI 1640 (Sigma) supplemented with 10% fetal calf serum (FCS), 2 mmol/L L-glutamine, and antibiotics (penicillin 100 U/mL, streptomycin 100 μg/mL). For the proliferation assay, cells (10^6/mL) were incubated in 96-well microplates (Costar, Cambridge, MA) in the presence of PHA (5 μg/mL) or with ATGs at the indicated concentrations. Cultures were maintained in a humid atmosphere at 37°C containing 5% CO_2 for the indicate time.

Immunofluorescence assays. Cells were washed with isotonic NaCl/Pi buffer containing 1% bovine serum albumin (BSA) and 0.2% NaN₃ (phosphate-buffered saline [PBS]/BSA/azide). Cells (5×10^5) were incubated with 10 μL labeled MoAbs for 30 minutes at 4°C. Then, after two washes in PBS/BSA/azide buffer, cells were fixed with 1% formaldehyde in PBS/BSA/azide buffer and analyzed by flow cytometry with a FACScan (Becton Dickinson, Pont de Claix, France). For intracellular analysis of Fas-L expression, cells were fixed with freshly prepared 2% paraformaldehyde in PBS and permeabilized by saponin (0.33%) (Sigma).

Measurement of apoptosis. After 3 days of culture, unstimulated or PHA-activated PBL were harvested. Dead cells were removed by centrifugation on a layer of Histopaque (Sigma), and viable cells were washed in HBSS. Viable cells (10^6/mL) were incubated in 96-well microplates in the presence of ATG or CH11 MoAb. After incubation, cell death was evaluated by three different techniques. Measurement of mitochondrial transmembrane potential by flow cytometry after 3,3'-dihexyloxacarbocyanine (DiOC₆) staining[22] and detection of phosphatidylserine expression by flow cytometry after addition of FITC-conjugated annexin V[23] were performed on the same suspensions at the indicated time. Nuclear apoptosis was assessed by fluorescence microscopy after staining with Hoechst 33342 (Sigma) at 10 μg/mL, following previously described methods.[24] Nuclear fragmentation or marked condensation of the chromatin with reduction of nuclear size, or both, were considered typical features of apoptotic cells. On the basis of these measurements, results were expressed either as percentage of apoptotic cells or as percentage of specific apoptosis according to the formula

$$\% \text{ Specific Apoptosis} = \frac{(\text{test} - \text{control}) \times 100}{(100 - \text{control})}$$

RNA isolation, reverse transcription, PCR amplification of Fas-L and mRNA, and quantification. Total cellular RNA was isolated from 5×10^6 cells, following the method of Chomczynski and Sacchi.[25] Reverse transcription of 1 μg RNA was performed using the first-stand

cDNA synthesis kit (Pharmacia Biotech, Orsay, France) in a total reaction volume of 15 μL. After 90 minutes at 37°C, the reaction was terminated by heating for 4 minutes at 95°C. PCR was performed in mixtures containing 1 μL cDNA derived from 10 ng total RNA, primers (100 ng of each; Eurogentech, Seraing, Belgium), 2.5 μL 10 × PCR buffer (Promega, Charbonnieres, France) containing 1.5 mmol/L MgCl₂, 0.05 mmol/L of each dNTP, and 0.5 U of Taq polymerase (Promega). Primers for Fas-L and Actin included Fas-L sense primer 5'CCA-TTT-AAC-AGG-CAA-GTC-CAA-CTC-3', Fas-L anti-sense primer 5'CAA-CAT-TCT-CGG-TGC-CTG-TAA-C-3', actin sense primer 5'GGG-TCA-GAA-GGA-TTC-CTA-TG 3', and actin anti-sense primer 5'GGTCTCAAACATGSATCTGGG-3'. These primers were designed to discriminate between the amplification of cDNA (low size PCR products) and contaminating genomic cDNA (high size PCR products). For each amplicon, 23 to 35 amplification cycles (1 minute at 94°C, 1 minute at 58°C, and 1 minute at 72°C) were performed with the PCR system 9600 (Perkin Elmer, Montigny-le-Bretonneux, France). Semi-quantitative evaluation of amplification products was performed as described by Morgan et al.[26] Briefly, each PCR product (15 μL) was electrophoresed on agarose gel (2%) stained with ethidium bromide and photographed using polaroid type 665 positive/negative film. The specificity of PCR reaction was confirmed by the expected size of the amplification products. The PCR signal intensities were quantitated by scanning the negative film using a Desktop Scanning Densitometer (PDI/Pharmacia Biotech, Saint-Quentin-Yvelines, France) and by evaluating the integrated trace optical density (OD) for each band using Quantity One Software (PDI/Pharmacia Biotech). The point for samples comparison in the exponential amplification range was selected by inspection from semi-logarithmic plots of OD versus cycle numbers. To correct for variations in the amount of input cDNA, results were expressed as the ratio Fas-L OD/actin OD at the point previously determined.

Complement-mediated lysis. Resting or PHA-activated PBMC were labeled with Na₂⁵¹CrO₄ for 2 hours at room temperature and washed twice. They were resuspended in medium at 2×10^6 cells/mL, and 100 μL of the suspension was added to round-bottomed microtiter plates containing 50 μL of an appropriate dilution of the antibody. After incubation for 10 minutes at room temperature, 50 μL of 40% fresh or heat-inactivated (56°C, 30 minutes) autologous serum (obtained from defibrinated blood) was added. The cell suspensions were incubated at 37°C for 30 minutes, then centrifuged at 100g for 2 minutes, and 100 μL of the supernatant was collected for measurement of released radioactivity. Controls without antibody were used to measure the spontaneous radioactivity release. The percentage of specific ⁵¹Cr release was calculated using the formula

$$\text{Specific Release} = \frac{(\text{test} - \text{spontaneous}) \times 100}{(\text{total} - \text{spontaneous})}$$

C1q binding. A total of 20 μL of ATGs or control Ig in PBS/BSA/azide was added to PBMC pellets (4×10^5) and incubated at 37°C for 30 minutes. After two washes in PBS, samples were separated in two and incubated at room temperature for 30 minutes in the presence of 50 μL of autologous serum or heat-inactivated (56°C, 30 minutes) serum as a control. After two washes, cells were incubated with 10 μL of polyclonal goat anti-C1q FITC antibody (1/50 Cappel, Durham, NC) at 4°C for 30 minutes. After two washes, cells were fixed with 1% formaldehyde in PBS/BSA/azide buffer and analysis performed on a FACScan flow cytometer.

Antibody-dependent cell cytotoxicity. Resting and PHA-activated PBMC were labeled with Na₂⁵¹CrO₄ for 2 hours at room temperature and washed twice. They were resuspended in medium at 1×10^6 cells/mL, and 50 μL of the suspension was added to round-bottomed microtiter plates containing 50 μL of an appropriate dilution of the antibody. After incubation for 10 minutes at room temperature, 100 μL
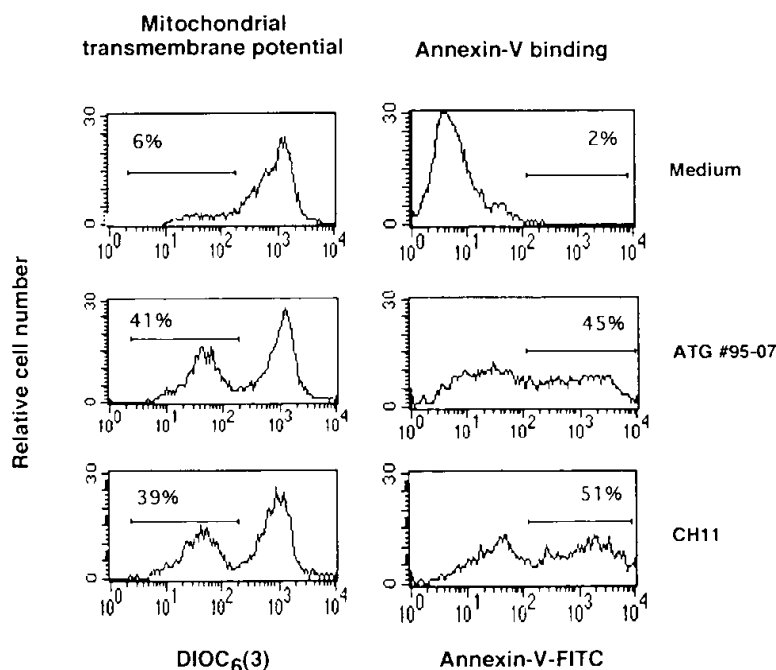
of effector cells (25 × 10⁶ cells/mL) was added. The cell suspensions were incubated at 37 C for 6 hours, then centrifuged at 100g for 2 minutes and 100 µL of the supernatant collected for measurement of released radioactivity as for complement-mediated lysis.
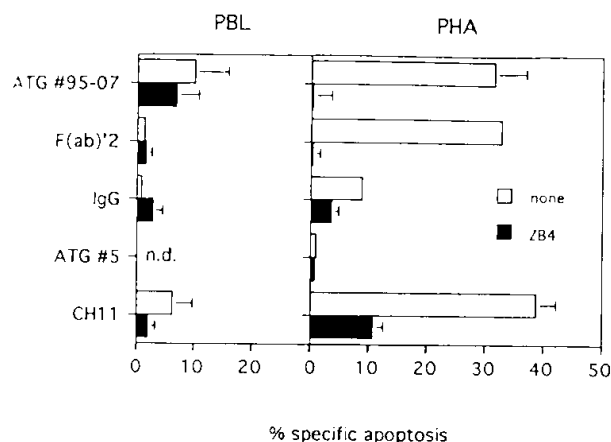
RESULTS

ATGs induce apoptosis of activated lymphoblasts. Knowing that ATGs could induce apoptosis of B-cell lines and to a lesser extent, T-cell lines,[27] we examined whether such mechanism could also take part in the elimination of peripheral T lymphocytes. Three-day PHA-activated PBL, as well as nonactivated PBL, were treated with ATG no. 95-07, F(ab')₂ fragments of ATG, anti-Fas MoAb CH11 as positive control, and normal rabbit IgG as negative control. Apoptosis was evaluated by DiOC₆[3] and annexin V staining (Fig 1) and by fluorescence microscopy after staining with Hoechst 33342 (Fig 2). The results showed that ATG no. 95-07 at nonmitogenic concentrations (10 µg/mL), their F(ab')₂ fragments, and the anti-Fas MoAb CH11 induced apoptosis of 30% to 40% of PHA-activated PBL, whereas resting PBL were not sensitive (Figs 1 and 2). Similar results were observed with ATG no. 1141 obtained from horse (data not shown). Interestingly ATG no. 5 containing CD18, CD11a, anti-β2m, and anti-HLA DR antibodies, but no CD3, CD2, and CD5 specificities, and which is not mitogenic at concentrations ranging from 1 to 1,000 µg/mL, did not induce apoptosis at 10 and 100 µg/mL (Fig 2; data not shown). Normal rabbit did not induce cell death of resting or activated PBL (Fig 2). Similar experiments were repeated with PBL activated by a 3-day culture period with PMA (10 ng/mL) plus ionomycin (500 ng/mL), PMA (10 ng/mL) plus OKT3 (100 ng/mL), or a mitogenic concentration of ATG no. 95-07 or no. 1141 (100 µg/mL). Whatever the activator used, the addition of ATGs (10 µg/mL) or F(ab')₂ fragments thereof resulted in specific apoptosis ranging from 20% to 50% (data not shown).

ATG-induced apoptosis is fully inhibited by an antagonist anti-Fas antibody. The apoptotic activity of ATGs was effective only on activated T cells, which express Fas and which are sensitive to Fas-mediated apoptosis[28]; we therefore studied whether ATG-induced apoptosis was dependent on Fas/Fas-L interaction. To this end, PHA-activated PBL were incubated for 1 hour with the antagonist anti-Fas MoAb ZB4, which blocks the interaction between Fas and Fas-L, before addition of ATG no. 95-07, ATG F(ab')₂ fragments or CH11 MoAb. As shown in Fig 2, ATG-induced apoptosis was completely blocked by ZB4, indicating that ATG-induced apoptosis of activated T cells required Fas/Fas-L interaction. This idea was re-enforced by the observation that simultaneous addition of ATG no. 95-07 (10 µg/mL) and CH11 resulted in the same percentage of apoptotic cells as with each antibody tested alone (data not shown). This result suggests that the same subset of activated T cells is the target of ATGs and anti-Fas antibodies. Furthermore, it shows that ATGs do not contain anti-Fas blocking antibodies, at least in sufficient amount to be detected in this assay.

ATGs induce Fas and Fas-L expression. In an effort to obtain further evidence for a possible role of Fas/Fas-L interaction in ATG-induced apoptosis, we examined whether ATGs would induce Fas-L expression in both resting and activated-PBL. To this end, PBL were first cultured in presence of a mitogenic concentration of ATG no. 95-07 (100 µg/mL) or PHA or medium alone for 3 days. After elimination of dead cells, preactivated PBL were then incubated for 6 hours with medium alone, ATG no. 95-07 at nonmitogenic (10 µg/mL) and mitogenic (100 µg/mL) concentrations or PHA, and induction of Fas-L mRNA was analyzed by RT-PCR. ATG no. 95-07 at either 10 or 100 µg/mL induced Fas-L mRNA expression by nonactivated and by preactivated-PBL (Fig 3). Similar experiments performed with freshly isolated PBL showed that ATG no.

Mitochondrial transmembrane potential

Annexin-V binding

Medium

ATG #95-07

CH11

DIOC₆(3)

Annexin-V-FITC

Relative cell number

Fig 1. Effect of ATGs on mitochondrial transmembrane potential and on phosphatidylserine expression. PBL were activated for 3 days in presence of PHA (5 µg/mL). After removal of dead cells, medium alone, ATG no. 95-07 (10 µg/mL), or CH11 anti-Fas MoAb (1 µg/mL) was added. After 12 hours, Δψm modifications were evaluated by staining with DiOC₆ (3). The expression of phosphatidylserine at the surface membrane was evaluated after 15 hours by measuring annexin-V binding. The percentage of cells with decreased mitochondrial potential membrane or increased expression of phosphatidylserine are indicated for each histogram. Results from one typical experiment among four showing similar percentages.
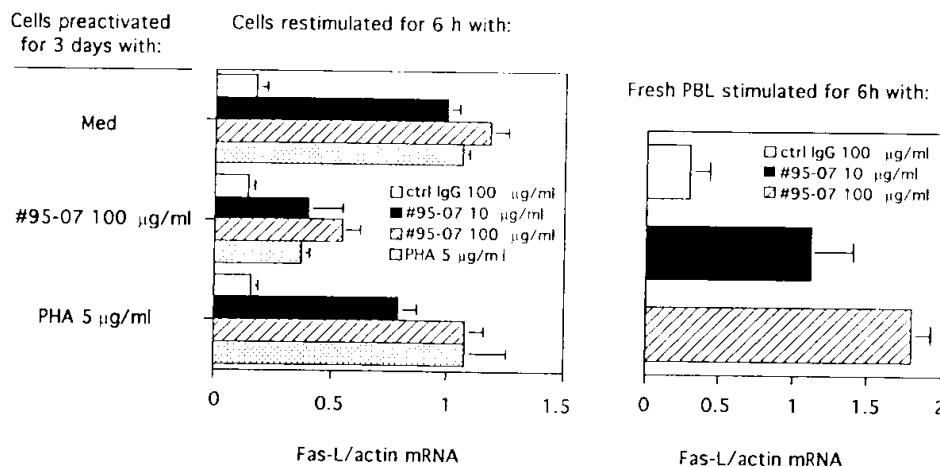
PBL   PHA

ATG #95-07

F(ab)'2

IgG   □ none
                             ■ ZB4

ATG #5   n.d.

CH11

0   10   20   0   10   20   30   40   50

% specific apoptosis

Fig 2. ATGs induce apoptosis of activated T lymphocytes. PBL were cultured in presence of medium alone or PHA (5 µg/mL) for 3 days. Dead cells were removed and viable cells were treated for 20 hours with ATG no. 95-07, F(ab)'$_2$ fragments of ATG no. 95-07, ATG no. 5 or normal rabbit IgG at 10 µg/mL or with the agonist anti-Fas MoAb CH11 at 1 µg/mL. Protection by the antagonist anti-Fas MoAb, was tested by pre-incubating PBL or PHA-activated cells for 1 hour with ZB4 MoAb at 2 µg/mL. The percentage of apoptotic cells was determined by fluorescent microscopy after staining with Hoechst 33342. Results are expressed as mean ± SEM of five different experiments or as mean of two experiments for ATG no. 5.

95-07 (10 and 100 µg/mL), but not control rabbit IgG, strongly induced Fas-L mRNA expression (Fig 3).

In parallel, surface expression of Fas and Fas-L molecules, but CD25 and CD69 activation markers as well, was analyzed by flow cytometry on PBL cultured in the presence of ATG no. 95-07 at 10 and 100 µg/mL for 1 to 3 days. At mitogenic concentrations (100 µg/mL), ATG no. 95-07 induced CD69, CD25, Fas, and Fas-L expression (Fig 4). Surface expression of Fas, CD69, and CD25 reached a maximum at day 2, and that of

Fas-L at day 1. At nonmitogenic concentrations (ie, 10 µg/mL), ATG no. 95-07 still induced expression of CD69, Fas, and Fas-L, but not that of the CD25 molecule, suggesting that, at low concentrations, ATGs drive lymphocytes into the G$_1$ phase of the cell cycle but did not allow them to progress to S phase because of the absence of CD25 expression. Interestingly ATG no. 5 at 100 µg/mL did not induce CD69 Fas and Fas-L expression (Fig 4), nor did it trigger apoptosis (Fig 2). Finally, these experiments were completed by intracellular staining of Fas-L in paraformaldehyde-fixed and saponin-permeabilized cells. The results indicate that ATG no. 95-07 (10 and 100 µg/mL) increased intracellular Fas-L in both resting and preactivated PBL, with a maximum on days 1 to 2 (Fig 4; data not shown). Histograms of fluorescence (Fig 4) show that a small subset of PBL is positive before activation, whereas after stimulation by ATG, most of the lymphocyte population becomes Fas-L positive.

Interference with the IL-2 pathway reduces ATGs-induced apoptosis. Knowing that IL-2 is required for acquisition of susceptibility to Fas-mediated apoptosis,[29,30] we analyzed the effect of immunosuppressive agents that interfere with the IL-2 pathway on ATG-induced cell death. PBL were cultured with PHA in the presence of CsA or FK506, which block IL-2 expression at a transcriptional level, or with RPM, which blocks IL-2 signaling. After 3 days, cells were treated with ATGs or F(ab')$_2$ fragments. The presence of CsA, FK506, or RPM, during T-cell activation, markedly decreased apoptosis mediated by ATG no. 95-07 or their F(ab')$_2$ fragments (Fig 5). In keeping with these results, we observed that addition of rIL-2 during the last 24 hours of cell culture, to PBL activated by PHA in the presence of CsA restored the sensitivity to ATG and F(ab')$_2$-induced apoptosis (Fig 5B). Conversely, the addition of interferon-γ (IFN-γ) restored T-cell proliferation,[29] but not the sensitivity to ATG-induced apoptosis. In agreement with previ-

Cells preactivated   Cells restimulated for 6 h with:
for 3 days with:

Med   Fresh PBL stimulated for 6h with:

                                             □ ctrl IgG 100 µg/ml
                                             ■ #95-07 10 µg/ml
                        □ ctrl IgG 100 µg/ml   ☑ #95-07 100 µg/ml
#95-07 100 µg/ml   ■ #95-07 10 µg/ml
                        ☑ #95-07 100 µg/ml
                        □ PHA 5 µg/ml

PHA 5 µg/ml

0   0.5   1   1.5   0   0.5   1   1.5   2

Fas-L/actin mRNA   Fas-L/actin mRNA

Fig 3. Expression of Fas-L mRNA induced by ATGs. (Left) PBL were cultured in presence of medium alone, ATG no. 95-07 (100 µg/mL) or PHA (5 µg/mL) for 3 days. Dead cells were removed, and viable cells were stimulated with normal rabbit IgG at 100 µg/mL, ATG no. 95-07 at 10 µg/mL and 100 µg/mL or PHA at 5 µg/mL for 6 hours. (Right) Freshly isolated PBL were stimulated with normal rabbit IgG at 100 µg/mL or ATG no. 95-07 at 10 µg/mL and 100 µg/mL for 6 hours. mRNA of each sample was amplified by RT-PCR as described in Materials and Methods with primers specific for actin or Fas-L. The number of amplification cycles selected within the exponential phase of PCR was 29 for actin and 32 for Fas-L. The PCR products were separated on 2% agarose gel and the PCR signal intensities were quantified by scanning the negative film. Results are expressed as the ratio of absorbance of Fas-L/absorbance of actin (mean ± SEM of three experiments).
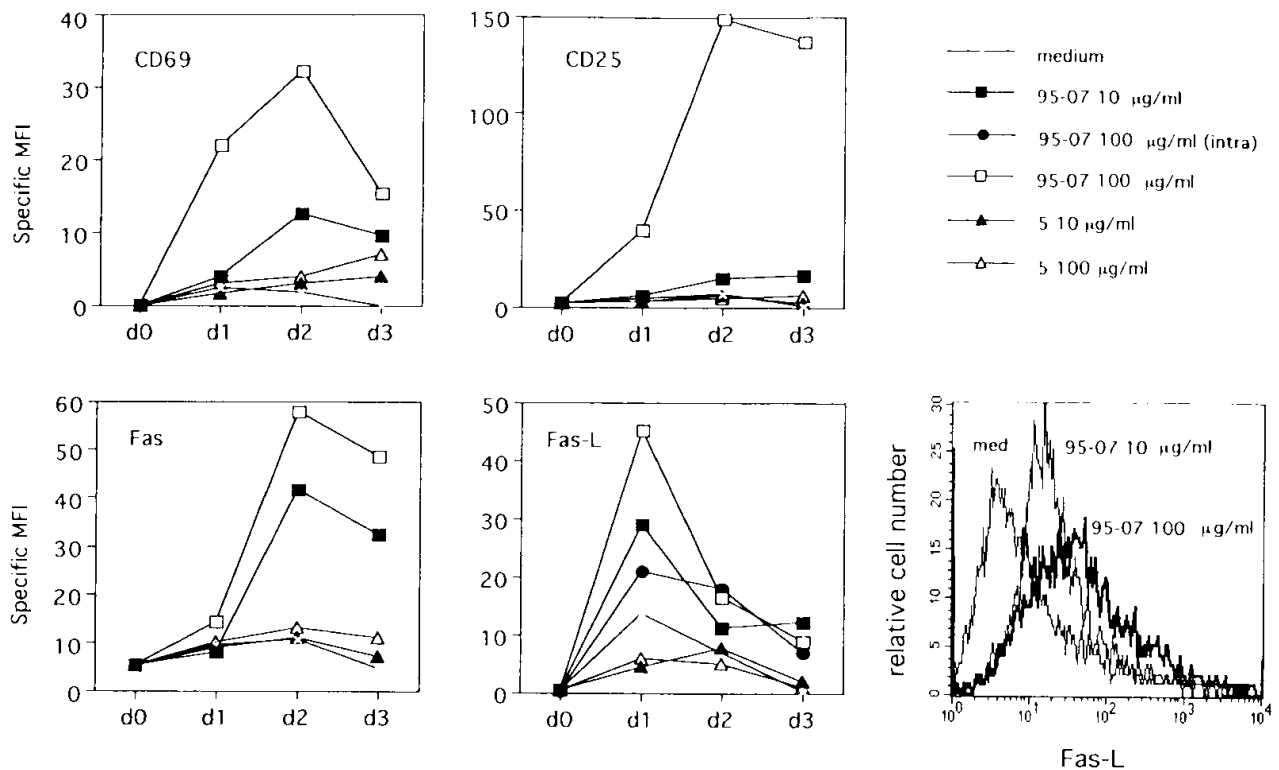
Fig 4. Effect of ATGs on CD69, CD25, Fas, and Fas-L surface expression. PBL were cultured in presence of medium alone or ATG no. 95-07 and ATG no. 5 at 10 μg/mL and 100 μg/mL for 3 days. At days 0, 1, 2, and 3, surface expression of CD69, CD25, Fas, and Fas-L was determined by cytofluorometry. In parallel, incorporation of [³H]TdR uptake during the last 8 hours of culture was measured (med 367 ± 41 cpm, ATG no. 5 10 μg/mL 391 ± 23 cpm, ATG no. 5 100 μg/mL 252 ± 26 cpm, ATG no. 95-07 10 μg/mL 532 ± 53 cpm, and ATG no. 95-07 100 μg/mL 11,500 ± 103 cpm). Histograms of Fas-L expression at day 1 are shown. Representative of four experiments with ATG no. 95-07 and of two with ATG no. 5.
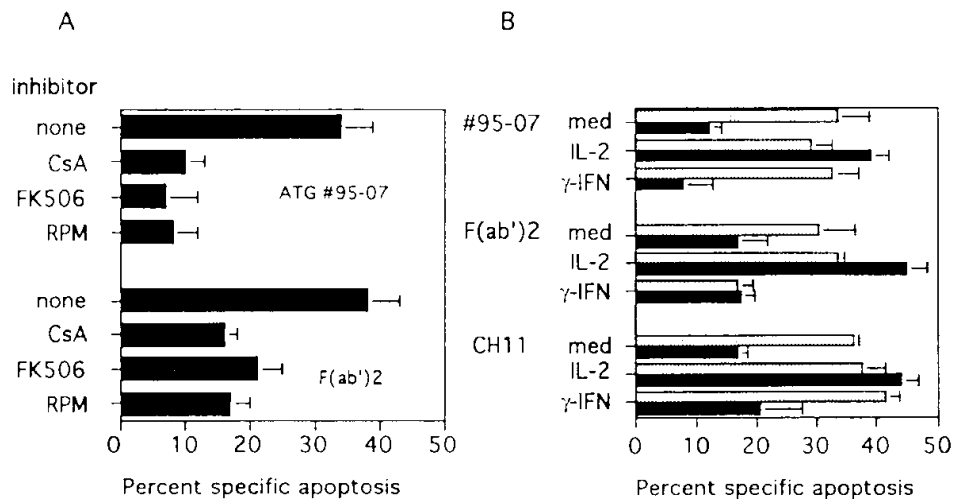
Fig 5. (A) Effect of immunosuppressive agents on ATG-mediated apoptosis. PBL were cultured for 3 days with PHA (5 μg/mL) and CsA (250 ng/mL), FK506 (10 nmol/L) or RPM (60 nmol/L) were added at the onset of the culture. Apoptosis was determined by fluorescence microscopy after staining with Hoechst 33342, 20 hours after treatment with ATG no. 95-07 or their F(ab′)₂ fragments at 10 μg/mL. (B) Effect of addition of exogenous IL-2 or IFN-γ. PBL were cultured for 3 days with PHA (5 μg/mL); medium alone (gray bars) or CsA (250 ng/mL) (black bars) were added at the onset of the culture. Recombinant IL-2 (25 U/mL) or rIFN-γ (500 U/mL) was added during the last 24 hours of activation. Apoptosis was determined by fluorescence microscopy after staining with Hoechst 33342, 20 hours after treatment with ATG no. 95-07, their F(ab′)₂ fragments at 10 μg/mL or the CH11 (1 μg/mL) MoAb. (Results are expressed as mean ± SEM of three different experiments).

% specific apoptosis
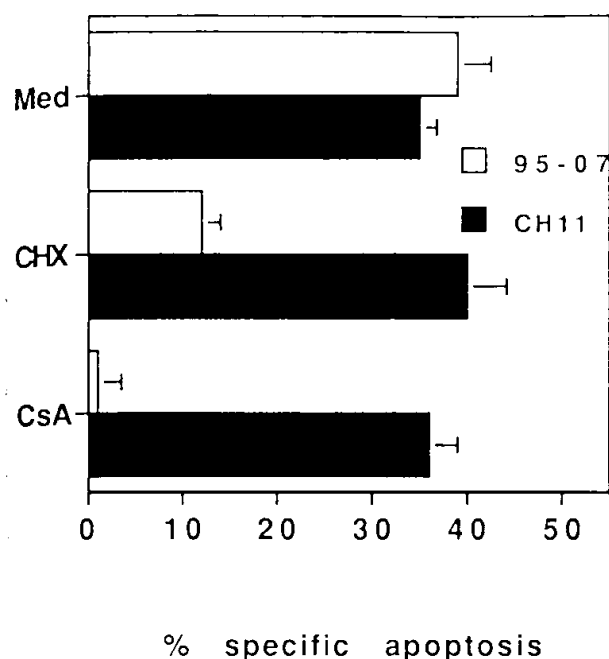
Fig 6. ATG-induced apoptosis is inhibited by CsA and requires protein synthesis. PBL were incubated for 3 days in the presence of PHA (5 µg/mL). Dead cells were removed and viable cells were incubated for 3 hours with CsA (250 ng/mL) or CHX (0.5 µg/mL) before treatment with ATG no. 95-07 (10 µg/mL) or CH11 (1 µg/mL). Apoptosis was determined by fluorescence microscopy after staining with Hoechst 33342. Results are expressed as mean ± SD of three different experiments.

ous reports,[29,30] similar effects were observed as regards sensitivity to Fas-mediated apoptosis (Fig 5B).

Furthermore, CsA and FK506 were described as strongly inhibiting Fas-L expression in murine T-cell hybridomas.[31] Thus, we have tested whether incubation of 3-day PHA-activated PBL with CsA, just before ATG treatment would interfere with ATG-induced apoptosis. A 3-hour preincubation of PHA-blasts with CsA or CHX inhibited ATG-induced cell death but did not interfere with apoptosis induced by the anti-Fas MoAb (Fig 6). These data suggest that immunosuppressive agents that interfere with the IL-2 pathway can prevent ATG-induced apoptosis by inhibiting either Fas-L synthesis or the acquisition of sensitivity to Fas-L–mediated cell death by activated T cells.

ATGs induce complement-mediated cytolysis at supramitogenic concentrations. Binding of human C1q was measured by incubation of PBL in the presence of ATGs and fresh human serum, followed by flow cytometry assessment of the amount of bound C1q per cell. Heat-inactivated human serum was used as control. Maximal binding was achieved at 1 mg/mL. At lower ATG concentrations, only rabbit, but not equine, ATG bound C1q (Fig 7). C1q binding was comparable between resting PBL and preactivated cells.

The ability of ATGs to induce resting or PHA-activated PBMC lysis was evaluated in the presence of an exogenous source of human complement. Minimal cytolysis was observed at 10 µg/mL with equine ATG, whereas maximal cytolysis was only achieved at very high concentrations (1 mg/mL) of ATGs. As a positive control of complement-mediated cytolysis, we used the CAMPATH-1M MoAb, which, in agreement with a
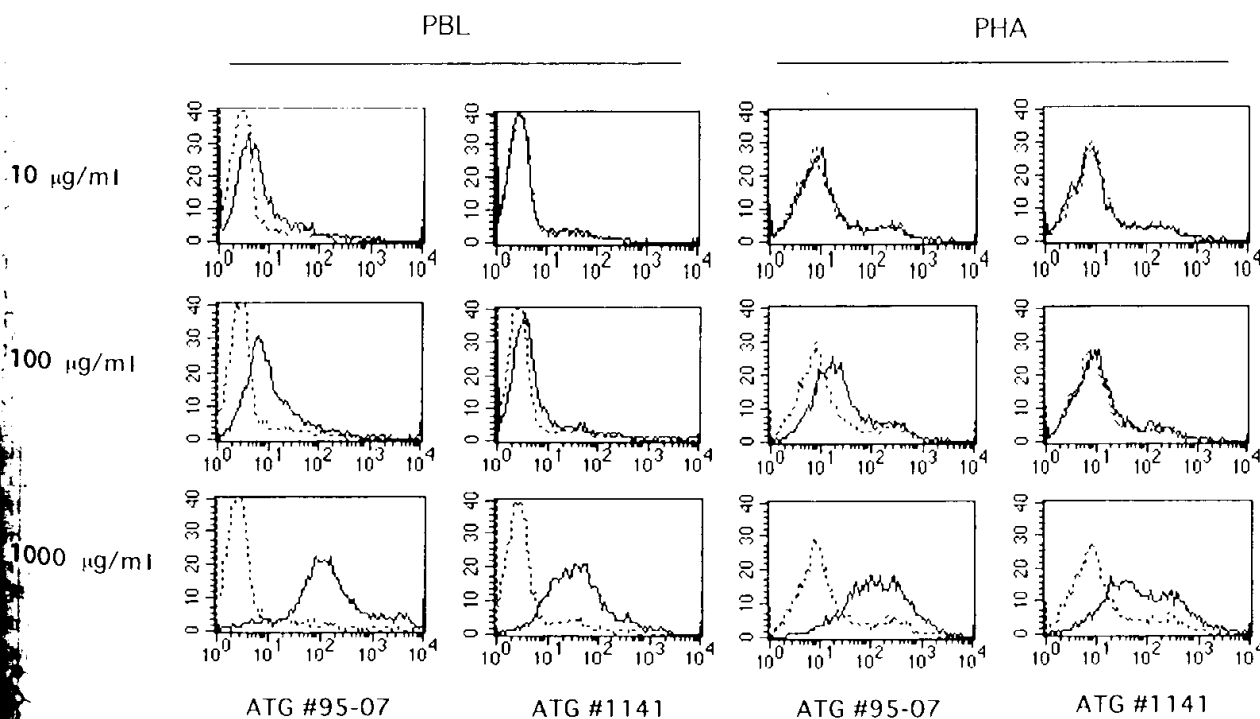
Fig 7. C1q binding to PBL or PHA-blasts sensitized with ATGs. PBL or PHA blasts were labeled with increasing amount of rabbit ATG (no. 95-07) or horse ATG (no. 1141) and then with autologous serum (solid line) or heat-inactivated serum as control (dashed line). C1q binding was detected by using FITC-goat anti-C1q polyclonal antibody and cell analyzed by flow cytometry as described in Materials and Methods. Representative of three independent experiments.
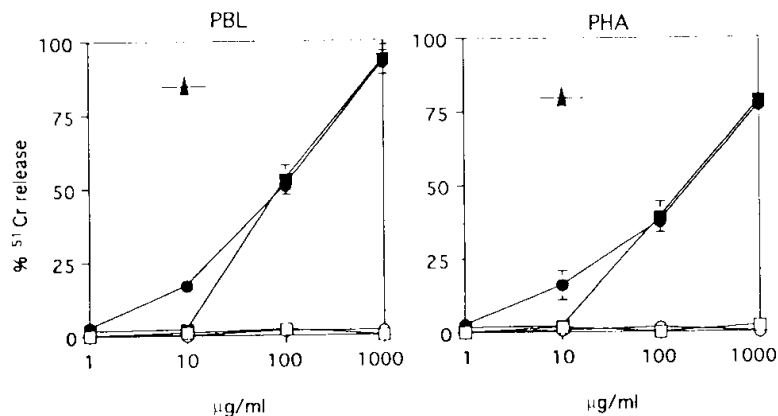
Fig 8. Complement-mediated lysis of PBMC versus PHA-Blasts. PBMC or 3-day PHA-activated PBMC were labeled with ^{51}Cr and incubated with rabbit ATG (no. 95-07) (■), horse ATG (no. 1141) (●), control horse (○) or rabbit IgG (□) or the anti-CD52 MoAb CAMPATH-1M (IgM) (▲) at the indicated concentrations, for 30 minutes at 37°C, in the presence of 10% autologous serum. Results are expressed as specific release as defined in Materials and Methods (mean ± SEM of three different experiments).

previous report,[32] induced about 80% lysis at 10 μg/mL. Of note, no difference was observed, whether ATGs were obtained from horse (no. 1141) or rabbit (no. 95-07), and whether resting or PHA-activated PBMC were used as target cells in the complement-dependent lysis assay (Fig 8).

ATGs induce antibody-dependent cell cytotoxicity at low concentrations. ATGs no. 95-07 and no. 1141 were tested for their ability to induce ADCC of both resting and PHA-activated PBMC. We observed that this effect was concentration dependent, with a maximal cytotoxicity at 1 μg/mL of ATG no. 95-07 and effective only when PHA-activated PBMC were used as target cells (Fig 9). As expected, the ADCC phenomenon was not observed with F(ab')₂ fragments of ATG no. 95-07 and was restricted to ATG from rabbit origin, because ATG no. 1141 did not induce cell lysis at concentrations ranging from 0.01 to 100 μg/mL.

DISCUSSION

Both horse antilymphocyte globulins and rabbit ATGs are still used in the treatment of severe aplastic anemia, organ allograft rejection, and graft-versus-host disease (GVHD), but their mechanisms of action remain largely unknown. A major common feature of ATG treatment is peripheral lymphocyte depletion,[1,4,5,33] which usually persists throughout the administration period and slowly reverses thereafter. Although not formally demonstrated in clinical studies, lymphocyte depletion is likely to account for the immunosuppressive activity of ATGs.[34] The present study addressed the mechanisms of peripheral lymphocytopenia, with special emphasis on the differential susceptibility of preactivated T cells (PHA blasts) versus nonactivated T cells to ATG-induced cell death. ATGs contain multiple antibody specificities with little batch-to-batch variability despite the use of different cell sources (thymocytes, T-cell lines, or B-cell lines) and different immunization protocols.[6-8] We therefore tested two ATG preparations of horse anti-human lymphocyte globulins (no. 1141) and rabbit anti-thymocyte globulins (no. 95-07) currently used in organ and bone marrow transplantation, as well as one horse ATG preparation (no. 5) previously used in kidney transplantation (selected because of its highly unusual lack of mitogenic activity related to the absence of demonstrable CD2 and CD3 specificities).[7] Horse anti-lymphocyte globulins are administered at 10 to 15 mg/kg/d,[33] and rabbit ATGs at 1.0 to 1.2 mg/kg/d, resulting in average serum levels of 0.5 mg/mL and 80 to 200 μg/mL, respectively.[5] These dosages have been selected mostly on empiric grounds, but individual dosage adjustment to maintain absolute T-cell numbers of 50 to 100 cells/μL did not result in a major decrease in daily doses.[33] It is worth noting that the 10-fold dosage difference between equine and rabbit ATGs is not paralleled by differences in either specific antibody titers (eg, CD2, CD3, CD4, CD8)[7] or in vitro functional properties such as T-cell activation[5,6,35,36] or B-cell apoptosis.[27]

Complement-dependent lysis is initiated by the binding of human C1q to ATG-coated cells. At low and intermediate ATG concentrations, C1q binding was demonstrable with rabbit ATG on both PBL and PHA blasts but remained borderline or not
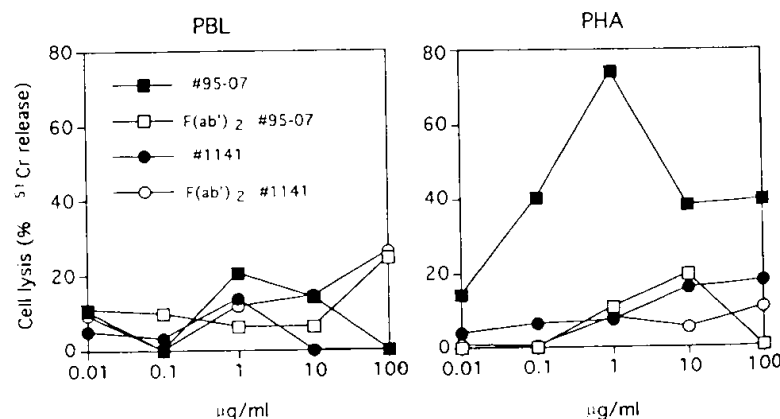

Fig 9. Antibody-dependent cell cytotoxicity of PBMC versus PHA-blasts. PBMC or 3-day PHA-activated PBMC were labeled with ^{51}Cr and incubated with rabbit ATG (no. 95-07), horse ATG (no. 1141), or their F(ab')₂ fragments at the indicated concentrations in presence of effector cells for 6 hours at 37°C. Results are expressed as specific release as in Fig 8. Representative of two independent experiments.

detectable with equine ATG (Fig 7). As shown with chimeric monoclonal antibodies of different isotypes, C1q binding may not be correlated with cell lysis.[17] Therefore, we used the highly sensitive chromium release assay to measure complement-dependent lysis. The data (Fig 8) indicate that equine and rabbit ATGs are equally effective on PBMC and PHA blasts, but only at high concentrations. In keeping with our observation, complement consumption, as measured by decreased serum CH50 activity, was recorded in some patients during equine ATG treatment, but never with rabbit ATG (Y. Lebranchu, personal communication, January 1997).

ADCC has been suggested as a possible mechanism of lymphocyte depletion by ATG.[1,5] NK cells present in peripheral blood are potent effectors of Fc receptor–dependent cell lysis. Our results indicate that only PHA blasts, but not PBMC, can be lysed through an ADCC mechanism, suggesting that rabbit ATGs could display some selectivity toward preactivated lymphocytes, should a similar mechanism operate in vivo. Equine ATG on the other hand was completely ineffective in this assay.

The major homeostatic mechanism that prevents lymphoid tissue hyperplasia despite repeated antigenic stimulations and T- or B-cell clonal expansion is activation-induced cell death (AICD) mediated by Fas/L-Fas interaction.[38] We therefore investigated the possible contribution of the Fas pathway in ATG-induced lympholysis. Fas-L is constitutively expressed in a variety of tissues, including immunologically privileged sites (eg, eye, Sertoli cells), some tumors,[39,40] and monocytes[41] and produced by a subset of T cells after repeated activation through the TCR/CD3 or CD2 pathways, or both.[42] Knowing the T-cell mitogenic properties of ATGs,[35,36] we were not surprised to observe that restimulation by ATGs of PBL preactivated by various mitogens, including ATGs themselves, triggered Fas-L gene expression (Fig 3). However, quite unexpectedly, ATGs were also found to induce Fas-L mRNA and protein expression in nonpreactivated PBL, even at low concentrations (10 µg/mL) sufficient to trigger CD69, but not CD25, expression and therefore remain below the mitogenic threshold (Fig 4). Although they express Fas receptors, these CD25 negative cells do not respond to IL-2 and therefore cannot become sensitive to Fas-dependent apoptosis, as discussed below. The fact that blocking Fas/Fas-L interaction completely suppressed ATGs-induced apoptosis (Fig 2) provides unequivocal evidence for a role of the Fas pathway in ATG-mediated lymphocyte cell death. Target cells for Fas-L should not only express Fas receptors that are rapidly induced upon activation, but should also become sensitive to Fas-mediated apoptosis, a property that is strictly dependent on an IL-2 signal.[29,30] Hence pharmacological interference with the IL-2 pathway in activated T cells, by the addition of CsA, FK506, or rapamycin, prevents Fas-positive cells from becoming sensitive to ATG- and to Fas-L- (or agonist anti-Fas antibody)–dependent apoptosis. CsA also inhibits Fas-L expression.[31,43] Therefore, concomitant administration of ATGs with any immunosuppressive agent that interferes with the IL-2 pathway (eg, CsA, FK506, rapamycin, CTLA-4-Ig, or CD25 antibodies) is likely to prevent Fas-dependent ATG-induced lymphocyte depletion. Furthermore, this mechanism of lymphocyte apoptosis may be impaired in clinical situations associated with high plasma levels of soluble Fas.

In conclusion, this in vitro study describes some of the

mechanisms that may account for lymphocyte depletion during ATG therapy. However, one should keep in mind that opsonization and subsequent phagocytosis by spleen, liver, and lung macrophages is likely to account for the massive and rapid lymphocytopenia observed with the current protocols. Nevertheless, other mechanisms should be considered, some of which could represent a therapeutic objective in the design of future protocols aimed at a more selective immunosuppression. Complement-dependent lysis does not discriminate between resting and preactivated T cells. Because it is achieved at high ATG concentrations, it may occur in treatment with horse ATG, but this is less likely with rabbit ATG. In this respect, the relevance of complement-dependent lymphocytotoxicity for the standardization of ATG preparations is questionable. Serum ATG concentrations achieved with current dosages are mitogenic for peripheral T cells. Hence, they could trigger Fas-L expression and induce sensitivity to Fas-L in the vast majority of T cells, unless CsA or FK506 that block these processes is administered concomitantly. An important finding of this study is that some ATG at low, submitogenic concentrations may trigger Fas-L expression, resulting in the selective death of preactivated, but not resting, lymphocytes. An ATG preparation (no. 5) lacking mitogenic activity, and with no demonstrable CD2 and CD3 specificities, was devoid of this property, suggesting that "lymphocyte activating" antibodies (eg, CD2, CD3) may be critical in achieving Fas-dependent apoptosis. Similarly, ADCC that also occurs at low rabbit ATG concentration selectively targets activated, but not resting, T cells. These properties could be used in protocols aiming at the selective elimination of in vivo activated T cells (eg, donor-specific alloreactive T cells in organ transplantation, recipient-specific T cells in GVHD), while sparing nonactivated T cells. Such protocols would require much lower doses than those currently used, in order to maintain serum ATG concentrations within a 10- to 20-µg/mL range, instead of 100 µg/mL. Their feasibility will be evaluated in the cynomolgus monkey and, depending on the outcome of these experiments, clinical trials may be considered.

ACKNOWLEDGMENT

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Non–Host-Reactive Donor CD8+ T Cells of Tc2 Phenotype Potently Inhibit Marrow Graft Rejection

By Daniel H. Fowler, Bernard Whitfield, Michael Livingston, Paul Chrobak, and Ronald E. Gress

Donor CD8+ T cells capable of host reactivity inhibit marrow graft rejection, but also generate graft-versus-host disease (GVHD). To evaluate whether the Tc1- and Tc2-type subsets of CD8 cells might inhibit rejection without host reactivity, we established an F1 into-parent murine bone marrow transplant model. Donor Tc1 and Tc2 cells were generated that preferentially secreted type I or type II cytokines; both subsets possessed potent cytolytic function, and clonally deleted host-type allospecific precursor CTL in vitro. B6 hosts receiving 950 cGy irradiation did not reject the donor marrow (F1 chimerism of 78.6%; n = 10), whereas hosts receiving 650 cGy rejected the donor marrow (3.8% chime-

rism; n = 8). At 650 cGy irradiation, the addition of Tc2 cells to the F1 marrow resulted in extensive F1 chimerism (70.8%) in 8 of 8 recipients; in contrast, alloengraftment was not consistently observed in mice receiving Tc1 cells or unmanipulated CD8 cells. Furthermore, when the preparative regimen was further reduced to 600 cGy, only hosts receiving the Tc2-type cells did not reject the F1 marrow. We conclude that Tc2 cells potently inhibit marrow graft rejection without inducing an alloaggressive response and that non–host-reactive Tc2 cells therefore facilitate engraftment across genetic barriers with reduced GVHD.

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THE HOST-VERSUS-GRAFT immune response, which results in graft rejection, is a primary limitation to the transfer of marrow across genetic barriers. The observation that recipients of T-cell–depleted transplants have a high rate of marrow failure[1] has led to the realization that donor T cells abrogate graft rejection; indeed, the presence of donor T cells in the marrow appears to be one of the primary determinants of whether alloengraftment occurs.[2] As such, administration of T-cell–enriched marrow is one approach to the facilitation of alloengraftment. However, in addition to preventing graft rejection, donor T cells generate an alloaggressive response against host antigens, which can result in graft-versus-host disease (GVHD). The donor CD8+ T-cell subset appears to be particularly capable of abrogating rejection[3]; however, CD8+ T cells also contribute significantly to the generation of GVHD.[4,5] Given this limitation, we have evaluated whether functional subsets of donor CD8 cells might prevent graft rejection with reduced GVHD.

Recently, the existence of cytokine-secreting subsets of cytotoxic CD8+ T cells has been demonstrated[6,7]: the Tc1 subset secretes the type I cytokines interleukin-2 (IL-2) and interferon-γ (IFN-γ), whereas the Tc2 subset secretes the type II cytokines IL-4, IL-5, and IL-10. Both cytokine-secreting subsets of CD8 cells possess cytolytic function, which has led to the Tc1/Tc2 terminology.[7] Such CD8 functional subsets appear to differentially mediate allogeneic responses; eg, we and others have observed that the Tc2 subset results in reduced GVHD.[8,9] Importantly, the Tc2 subset can also mediate a graft-versus-leukemia (GVL) effect.[8,10] These results suggest that Tc2 cells might represent a CD8 population capable of mediating beneficial allogeneic responses (such as the mediation of GVL effects or the abrogation of graft rejection) with reduced detrimental effects (less severe GVHD). In light of these observations, we have compared the Tc1 and Tc2 subsets of CD8+ T cells for their ability to prevent marrow rejection.

To study the engraftment effects of the Tc1 and Tc2 subsets of donor CD8 cells independent of their GVHD effects, we have established an F1 into-parent model of graft rejection (B6C3F1 bone marrow into sublethally irradiated B6 hosts). In this type of rejection model, donor CD8 cells share the haplotype of the parental host and thus do not induce an alloaggressive reaction against the host; T-cell–mediated facilitation of engraftment in such models has been attributed to a veto effect.[11] In the veto

effect, host-type precursor CTL capable of mediating rejection are clonally deleted by cytotoxic donor cells that express the alloantigens present on the marrow graft[12]; in addition, the donor cells mediating the clonal deletion are nonreactive to host antigens. The definition of veto cells is therefore a functional one, and multiple cell types have been observed to possess veto-type activity in the setting of murine allogeneic bone marrow transplantation, including bone marrow-derived natural killer cells[13] and CD4+[14] and CD8+[3,14] T cells. Previous experiments have demonstrated that the perforin/granzyme pathway of cytolysis is important in the mediation of the veto effect.[12] Thus, in light of studies that indicate that the Tc2 subset of CD8+ T cells preferentially uses the perforin/granzyme pathway,[15] we hypothesized that non–host-reactive Tc2 cells would prevent marrow rejection.

In this study, we compared the Tc1 and Tc2 subsets of donor CD8+ T cells for their ability to facilitate alloengraftment and have determined that the Tc2 subset potently inhibits marrow graft rejection. By using an F1 into-parent model, we have shown that Tc2-mediated abrogation of rejection can occur independent of an alloaggressive response. The administration of non–host-reactive Tc2 cells therefore represents a strategy for abrogating rejection with reduced GVHD and thus may allow for the transfer of marrow across genetic barriers.

MATERIALS AND METHODS

In vitro generation of donor CD8+ T cells of Tc1 and Tc2 phenotype. Spleen cells from donor B6D2F1 mice (C57BL/6 × DBA/2, H-2b/d; obtained from Frederick Cancer Research Facility [Frederick, MD] and

used at 8 to 16 weeks of age) were harvested, lysed in Tris-ammonium chloride buffer (Biofluids, Rockville, MD), and brought to a concentration of 4×10^7 cells/mL in RPMI 1640 (Life Technologies, Grand Island, NY) containing 10% fetal calf serum (FCS; HyClone, Logan, UT). This splenic single-cell suspension was enriched for T cells (goat antimouse H and L bioparticles; PerSeptive Diagnostics, Cambridge, MA) and then enriched for CD8 cells by complement treatment (rabbit low-tox; Cedarlane, Hornby, Ontario, Canada) after incubation with anti-CD4 (supernatant from clone RL172/4[16]). The CD8-enriched spleen cells were then resuspended at 1×10^6/mL and cocultured at a ratio of 1:4 with irradiated (3,000 cGy) whole spleen cells from B6C3F1 mice (C57Bl/6 × C3H/HeN, H-2^b/k).

The coculture was performed in 75-cm² flasks (Costar, Cambridge, MA) in 40 mL of RPMI 1640 supplemented with 10% FCS, sodium pyruvate (1%), nonessential amino acids (1%), 2-ME (5×10^{-5} mol/L), penicillin (0.5%), streptomycin (0.5%), and N-acetyl cysteine (Sigma Chemical Co, St Louis, MO; 10 mmol/L, pH adjusted to 7.2). All cocultures received recombinant human IL-2 at 40 Cetus units (CU)/mL (kindly provided by Dr Martin Giedlin, Chiron Therapeutics, Emeryville, CA) and recombinant human IL-7 (20 ng/mL; Peprotech, Rocky Hill, NJ) on days 0 and 2. Tc1 cultures were supplemented (day 0) with recombinant murine IL-12 (20 U/mL; kindly provided by Dr Stan Wolf, Genetics Institute, Cambridge, MA) and recombinant human transforming growth factor β-one (TGF-β; 10 ng/mL; R&D Systems, Minneapolis, MN), whereas Tc2 cultures were supplemented (day 0) with recombinant murine IL-4 (1,000 U/mL; Peprotech). On day 5 of culture, Tc1 and Tc2 flasks were harvested, brought to a final concentration of 0.5×10^6/mL in 40 mL of fresh media, and restimulated with irradiated (3,000 cGy) B6C3F1 spleen cells at a ratio of 1:4. At the time of restimulation, IL-2 (40 U/mL) and IL-7 (20 ng/mL) were added to both Tc1 and Tc2 cultures.

Flow cytometric (FCM) evaluation of Tc1/Tc2 surface phenotype. To evaluate the Tc1 and Tc2 populations for cell surface phenotype, aliquots from the CD8 cultures were harvested on day 7, washed, and resuspended in FCM media consisting of Hanks' balanced salt solution (HBSS; Life Technologies) supplemented with 0.5% bovine serum albumin (BSA; Sigma) and 0.1% azide. Cells were first incubated with unlabeled anti-Fc receptor (2.4G2; PharMingen, San Diego, CA) and then stained with anti-CD4 fluorescein isothiocyanate (FITC) and anti-CD8 phycoerythrin (PE) (Becton Dickinson Immunocytometry Systems [BDIS], Mountain View, CA); negative control stains consisted of Leu8 FITC and Leu4 PE. Cells were also stained with anti-CD69 PE (PharMingen). Two-color flow cytometry was performed on a FACSort (BDIS) using LYSIS II software. Five thousand to 10,000 live events were acquired for analysis; dead cells were gated out on the basis of propidium iodide staining.

Cytokine secretion profiles of Tc1 and Tc2 populations by enzyme-linked immunosorbent assay (ELISA). On day 7 of culture, aliquots from Tc1 and Tc2 cultures were harvested, brought to a final concentration of 0.5×10^6/mL, and stimulated in 24-well plates (Costar) with either syngeneic B6D2F1 or semiallogeneic B6C3F1 spleen cells (irradiated 3,000 cGy; 1:4 ratio). Supernatants were harvested after 24 hours and tested in two-site ELISAs using commercially available reagents (purified and biotinylated anti-cytokine antibody pairs; PharMingen). Cytokine levels were calculated by reference to standard curves constructed on supernatants containing known amounts of recombinant cytokine.

Evaluation of Tc1/Tc2 cytolytic function. On day 7 of culture, aliquots from Tc1 and Tc2 cultures were harvested and tested for their ability to lyse the allogeneic tumor line P210[17] (H-2^k; myeloid line transfected with the bcr/abl oncogene; kindly provided by Dr James Griffin, Dana-Farber Cancer Institute, Boston, MA). The syngeneic control target EL-4 (H-2^b; American Type Tissue Culture TIB 39) was used to determine allospecificity of cytolytic function; as a positive control for this syngeneic target. CD8⁺ T cells from DBA mice were

stimulated under Tc1 and Tc2 conditions using spleen cells from mice (H-2^b) as stimulator cells. Standard chromium-release assays performed, with calculation of the percentage of specific lysis.[18]

In vitro assay of Tc1- and Tc2-mediated deletion of precursor Using a previously described in vitro model of veto cell function, tested the Tc1 and Tc2 populations for their ability to clonally d allospecific precursor CTL. In this model, a mixed lymphocyte rea was established using a 10:1 mixture of responder spleen cells 1 C57Bl/6(H-2^b) and C57Bl/6 transgenic mice (2C mice; CD8⁺ T transgenic at the TCR locus for L^d allospecificity[19]). Responder (4×10^6) were stimulated in 24-well plates (Costar) with irradi spleen cells (2,500 cGy) from DBA/2 mice (H-2^d). Expansion of allospecific CD8⁺ T cells was monitored by daily cell count determi tions and flow cytometry (the transgenic TCR was identified by f cytometry after staining with directly FITC-labeled 1B2 antib specific for the transgenic TCR[19]). In this culture system, transge CD8⁺ T cells undergo an approximate 10-fold expansion between d 2 and 3 of the MLR. To evaluate the ability of CD8 functional T subsets to mediate veto activity in vitro, Tc1 and Tc2 populations w generated (as described above) and added to the transgenic cultures day 2; on day 3, cell counting and flow cytometry was performed determine transgenic CTL yield. The Tc1 and Tc2 populations w generated from CD8⁺ T cells from B6D2F1 mice and thus shared t H-2^b haplotype with the transgenic CTL and shared the H-2^d haploty with the irradiated stimulator cells. As such, Tc1- or Tc2-mediat deletion of the transgenic CTL may occur by a veto-type mechanism.

F1 into-parent transplantation model. Parental B6 mice receive total body irradiation (^137Cs γ radiation source, 101 cGy/min; Gamm Cell 40; Atomic Energy of Canada, Ltd, Ottawa, Ontario, Canada depending on the particular experiment, the radiation dose varied fro 600 to 950 cGy. Four to 6 hours after irradiation, all mice received a intravenous injection of 1×10^7 T-cell–depleted (TCD) bone marrow cells from B6D2F1 mice (antibody/complement depletion using th anti–T-cell monoclonal HO-13-2[20]). Control mice received only th TCD marrow, whereas recipients in other groups also received a separate intravenous injection of 1×10^7 CD8⁺ T cells of donor B6D2F1 origin; such CD8⁺ T cells consisted of either in vitro generated CD8⁺ T cells of Tc1 or Tc2 phenotype (harvested from flasks on day 7 of culture) or uncultured B6D2F1 CD8⁺ T cells (prepared by T-cell enrichment and anti-CD4/complement treatment, as described above).

Evaluation of donor chimerism posttransplant by flow cytometry. Donor cell chimerism of transplant recipients for each experiment was determined by flow cytometry at approximately 1 month posttransplant and again after 3 months posttransplant. Heparinized peripheral blood was obtained from the retro-orbital sinus, and lymphocytes were isolated by density centrifugation (Cellsep; 1.077 specific gravity; Larex, Inc, St Paul, MN) and subsequently washed in FCM media. Cells were incubated with unlabeled anti-Fc receptor antibody (2.4G2; PharMingen) and stained with anti-H-2k^b FITC and anti-H-2k^d PE (PharMingen); cells were also stained with isotype control antibodies to define background staining. Lymphocytes taken from untreated B6 and B6D2F1 mice were used to define postive and negative quadrants. Other stains used to evaluate lineages of engraftment were anti-CD19 FITC, anti-granulocyte FITC, and anti-Thy 1.2 FITC (all from Phar-Mingen).

Statistical methodology. P values were obtained using the two-sided matched-rank analysis of Wilcoxin; values less than .05 were considered statistically significant.

RESULTS

Phenotyping of in vitro-generated donor CD8⁺ T cells of Tc1 and Tc2 phenotype. After 7 days, cells from the Tc1 and Tc2 cultures were phenotyped by flow cytometry. Both Tc1 and Tc2 culture conditions resulted in a population that was greater than

90% CD8+, with less than 2% contaminating CD4+ cells. Similar to results we have reported previously.[8] cells from the Tc2 culture had lower surface CD8 expression relative to the Tc1 culture (mean fluorescence intensity of CD8 expression for Tc2 cells was 3,662, whereas Tc1 cell mean fluorescence intensity of CD8 expression was 5,131). Also, expression of surface CD69 was measured to compare the Tc1 and Tc2 cultures for their activation status[21]: the majority of cells (>85%) in both Tc1 and Tc2 cultures were positive for CD69, indicating that both populations were similarly activated at the time of their in vivo evaluation (day 7 of culture).

On day 7 of culture, cells from the Tc1 and Tc2 cultures were harvested and evaluated for cytokine phenotype. To evaluate the allospecific cytokine secretion pattern, cultured CD8 cells were restimulated with either syngeneic B6D2F1 spleen cells or semiallogeneic B6C3F1 cells; cytokine secretion was allospecific, because stimulation of Tc1 and Tc2 cells with syngeneic spleen cells did not result in significant cytokine production above the detection limits of the assays (IL-2 assay, 0.8 CU/mL; IFN-γ, 1.0 IU/mL; IL-4, 20 pg/mL; IL-5, 320 pg/mL; and IL-10, 40 pg/mL). In response to restimulation with the third-party alloantigen (H-2[k]), cells from the Tc1 culture secreted the type I cytokines IL-2 (10.9 CU/mL) and IFN-γ (152 IU/mL), but did not secrete the type II cytokines. In marked contrast, cells from the Tc2 culture secreted the type II cytokines IL-4 (204 pg/mL), IL-5 (3,193 pg/mL), and IL-10 (1,510 pg/mL) and secreted reduced levels of the type I cytokines IL-2 (1.2 CU/mL) and IFN-γ (19.9 IU/mL).

Cytolytic function of the Tc1 and Tc2 cultures was evaluated in chromium release assays using the allogeneic tumor target, P210: this target shares the alloantigen (H-2[k]) used for the in vitro CD8 generation. As Fig 1 shows, CD8+ T cells secreting either type I or type II cytokines were similarly effective in their lysis of the allogeneic P210 target. In contrast, the Tc1 and Tc2 populations mediated only nominal lysis of the syngeneic EL-4 target; the ability of the EL-4 control target to be lysed was confirmed by generating Tc1- and Tc2-type effector cells of anti-H-2[b] specificity (both populations showed greater than 65% specific lysis of the EL-4 target at a 30:1 E:T ratio). Thus, the Tc1 and Tc2 populations were allospecific both in their cytokine secretion and cytolytic function. As such, the in vitro culture methodology was effective in generating allospecific CD8+ donor T cells of Tc1 and Tc2 phenotype.

Both Tc1- and Tc2-type populations effectively delete precursor CTL in vitro. Graft rejection is mediated in part by radioresistant allospecific CTL[22-25]; the clonal deletion of such CTL may be one mechanism whereby donor T cells abrogate graft rejection. A model has been developed to evaluate the ability of cytotoxic cells to clonally delete allospecific CTL in vitro by a veto-type mechanism[12]; using this model, we compared the Tc1 and Tc2 populations for their ability to clonally delete precursor CTL. In this model, the fate of allospecific precursor CTL is determined by measuring the flow cytometric expression of the transgenic TCR expressed by these CTL. It is important to note that, similar to the in vivo graft rejection model, this in vitro model is a one-way alloreactive system; ie, the transgenic precursor CTL can recognize the stimulator cells and the Tc1 or Tc2 populations, but the Tc1 and Tc2 cells are syngeneic relative to the precursor CTL. Figure 2
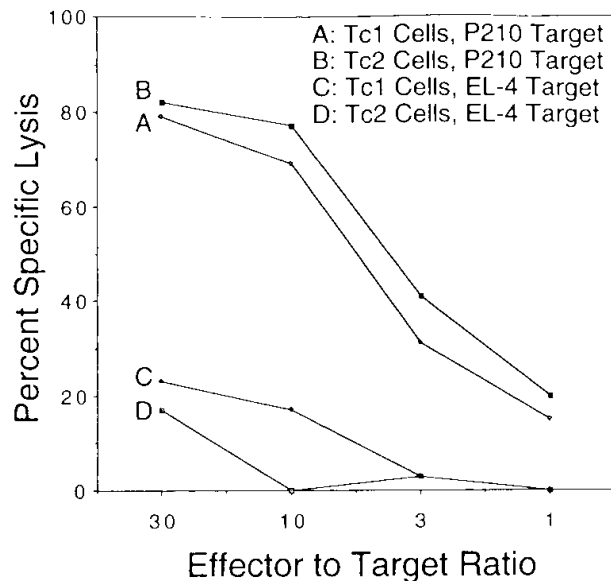
Fig 1. Cytolytic function of the Tc1 and Tc2 populations. CD8+ T cells from B6D2F1 donor mice were stimulated in vitro with irradiated spleen cells from B6C3F1 mice under Tc1 or Tc2 conditions, harvested on day 7 of culture, and plated in a standard 4-hour chromium-release assay at the stated E:T ratios with the allogeneic tumor target, P210 (H-2[k]), or the syngeneic tumor target, EL-4 (H-2[b]). Each data point was performed in triplicate, with less than 5% standard deviation for each point.

shows that both Tc1 and Tc2 cells were similarly effective in their ability to delete the allospecific precursor CTL population: the addition of 1×10^6 Tc1 or Tc2 cells on day 2 of the MLR resulted in an approximate one log reduction in transgenic CTL number by day 3 of culture.

F1 CD8+ T cells of Tc2 phenotype potently inhibit marrow graft rejection. Having generated CD8+ T cells of both Tc1 and Tc2 phenotype from F1 donors, we next evaluated their effect on F1 marrow engraftment in an F1 into-parent model of graft rejection. B6 mice were sublethally irradiated and subsequently received marrow from B6D2F1 mice; control mice received only the F1 marrow, whereas other treatment groups received additional donor CD8+ T cells of Tc1 or Tc2 phenotype. Figure 3 shows the flow cytometry result for determination of F1 chimerism at both day 30 and day 90 posttransplant. Irradiation of the B6 hosts with a high dose of irradiation (950 cGy) resulted in F1 marrow engraftment (the range of F1 chimerism was 56% to 76% at day 30 and 92% to 95% at day 90). In contrast, B6 hosts that were irradiated at a lower dose (650 cGy) displayed nearly complete host-type chimerism by day 90 posttransplant (<2% F1 chimerism), thus indicating rejection of the F1 marrow. In this experiment, hosts prepared with 650 cGy irradiation that received marrow enriched with F1 CD8+ T cells of Tc1 phenotype also rejected the F1 marrow (<3% chimerism in all recipients). In marked contrast, hosts prepared with 650 cGy irradiation that received additional CD8+ T cells of Tc2 phenotype had predominately donor-type chimerism (the range of F1 chimerism was 51% to 66% at day 30 and 82% to 91% at day 90). Other flow cytometry data (not shown) indicated that the chimerism occurred in the T-cell, B-cell, and granulocyte lineages. This experiment therefore
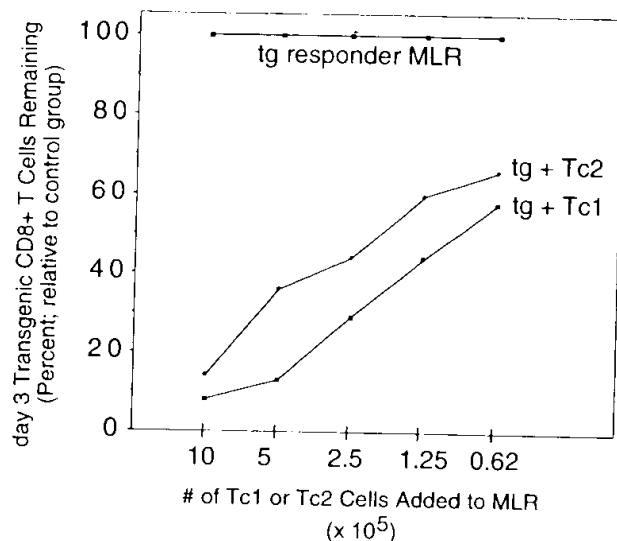
Fig 2.   Both Tc1 and Tc2 populations clonally delete precursor CTL in vitro by a veto-type mechanism. A mixed lymphocyte reaction was established in 24-well plates using a 10:1 mixture of responder spleen cells from C57Bl/6(H-2^b) and C57Bl/6 transgenic mice (2C mice; CD8$^+$ T cells are transgenic at the TCR locus for L^d allospecificity) and stimulator spleen cells from DBA/2 mice (H-2^d). The yield of transgenic CD8$^+$ T cells in the MLR (tg responder MLR) was calculated by determination of cell counts and transgene percentage (transgenic TCR was identified by flow cytometry after staining with FITC-labeled 1B2 antibody); in this system, the transgenic CD8 population expands appoximately 10-fold between days 2 and 3 of the MLR. To evaluate the ability of Tc1- and Tc2-type cells to clonally delete this transgenic population by a veto mechanism, CD8$^+$ T cells from B6D2F1 donor mice were stimulated in vitro with irradiated spleen cells from B6C3F1 mice under Tc1 or Tc2 conditions, harvested on day 7 of culture, and added to the transgenic MLR at the indicated numbers per well (tg + Tc1 and tg + Tc2) on day 2 of the MLR. The yield of transgenic CD8 cells was then determined on day 3 of the MLR.

indicated that the Tc2-type cells had a marked ability to prevent acute marrow graft rejection and that Tc2-mediated facilitation of engraftment resulted in long-term, stable marrow engraftment.

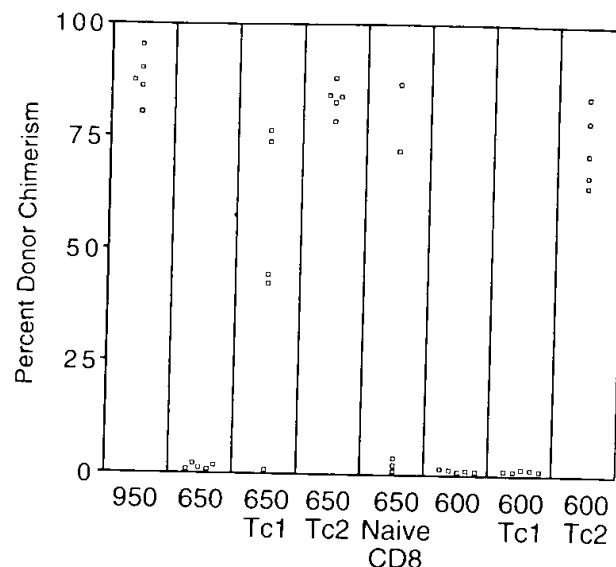To further evaluate the effect of the Tc1 and Tc2 populations

Fig 4.   Tc2-type donor CD8$^+$ T cells are enriched in their ability to abrogate marrow graft rejection. Host B6 (H-2^b) mice were irradiated at 950, 650, or 600 cGy; all mice received 1 × 10^7 TCD bone marrow cells from B6D2F1 (H-2$^{b/d}$) donor mice. Engraftment control mice (950/−) and rejection control mice (650/−) received only the donor bone marrow at the time of transplantation; other groups received additional in vitro-generated donor CD8$^+$ T cells (1 × 10^7 cells) of Tc1-type (650/Tc1, 600/Tc1) or Tc2-type (650/Tc2, 600/Tc2) or additional unmanipulated donor CD8 cells (650/naive CD8). Each treatment group consisted of 5 mice. Peripheral blood lymphocytes were isolated on day 41 posttransplant and stained with H-2^b FITC (common to both donor and host cells) and H-2^d PE (specific for donor cells); the percentage of donor and host chimerism was then determined by flow cytometry.

on F1 marrow engraftment, B6 host mice were irradiated (950, 650, or 600 cGy) and received injections of either F1 marrow alone or marrow and F1 CD8 cells that were cultured in vitro under Tc1 or Tc2 conditions; a separate treatment group received F1 marrow and unmanipulated F1 CD8$^+$ T cells (naive CD8). As Fig 4 shows (chimerism results on day 41 posttransplant), hosts receiving 950 cGy irradiation displayed nearly
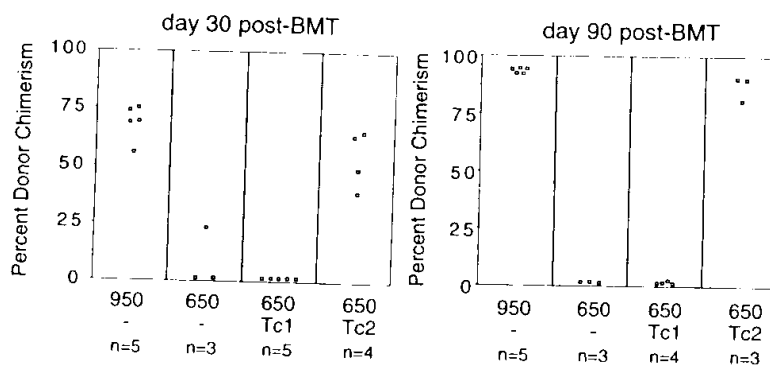

Fig 3.   Tc2-type donor CD8$^+$ T cells abrogate marrow graft rejection. Host B6 (H-2^b) mice were irradiated at 950 or 650 cGy; all mice received 1 × 10^7 TCD bone marrow cells from B6D2F1 (H-2$^{b/d}$) donor mice. Engraftment control mice (950/−) and rejection control mice (650/−) received only the donor bone marrow at the time of transplant; other groups received additional donor CD8$^+$ T cells (1 × 10^7 cells) of Tc1-type (650/Tc1) or Tc2-type (650/Tc2). Peripheral blood lymphocytes were isolated on days 30 and 90 posttransplant and stained with H-2^b FITC (common to both donor and host cells) and H-2^d PE (specific for donor cells); the percentage of donor and host chimerism was then determined by flow cytometry. Each data point represents the donor chimerism result for an individual animal.

0 600 600
Tc1 Tc2
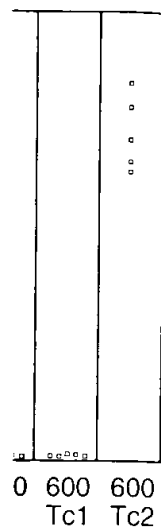
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complete F1 engraftment, whereas hosts receiving 650 cGy irradiation uniformly rejected the F1 marrow. At 650 cGy of host irradiation, mice receiving the F1 marrow and CD8⁺ T cells of Tc1 phenotype displayed a variable level of F1 engraftment (F1 chimerism of 0.6%, 42%, 44%, 73%, and 76%); thus, in contrast to the experiment shown in Fig 3, donor CD8 cells of Tc1-type were capable of abrogating the marrow graft rejection response. The administration of unmanipulated F1 CD8 cells also did not result in consistent F1 engraftment (F1 chimerism of 0.7%, 3%, 8%, 71%, and 86%). Thus, at 650 cGy host irradiation, donor T cells of naive or Tc1 phenotype partially abrogated the graft rejection response. In contrast, mice receiving F1 marrow and Tc2-type CD8 cells had a high level of F1 chimerism in 5 of 5 recipients at the 650 cGy dose of irradiation (F1 chimerism of 78%, 82%, 83%, 84%, and 88%). When the results shown in Figs 3 and 4 are pooled (650 cGy host irradiation), the Tc2 population was found to abrogate the rejection of TCD marrow (+Tc2 > marrow alone; $P = .01$) and found to prevent marrow rejection more potently than Tc1-type cells (+Tc2 > +Tc1; $P = .008$).

As Fig 4 shows, mice irradiated at 600 cGy that received marrow supplemented with Tc2-type cells were uniformly engrafted with the F1 marrow (F1 chimerism in 5 of 5 recipients; 64%, 66%, 71%, 78%, and 84% F1 chimerism); in contrast, 5 of 5 Tc1 recipients (600 cGy irradiation) displayed less than 1% F1 chimerism. Similar chimerism results were also obtained in this experiment at 161 days posttransplant (not shown). This experiment confirms that donor CD8⁺ T cells of Tc2 phenotype are enriched for an ability to prevent marrow graft rejection and shows that Tc2 cells are capable of facilitating alloengraftment in the setting of less intensive host preparative regimens.

DISCUSSION

In this report, we have evaluated the effect of donor CD8⁺ T cells of Tc1 and Tc2 phenotype on marrow graft rejection and have determined that the Tc2 subset is particularly potent in its ability to facilitate alloengraftment. The Tc2 subset, which possessed cytolytic function and secreted type II cytokines, prevented the rejection of MHC-disparate marrow in sublethally irradiated hosts and allowed for a significant reduction in intensity of the host preparative regimen. Because we used an F1 into-parent model in these studies, our results demonstrate that Tc2-mediated abrogation of rejection does not require an alloaggressive response against the host. These findings thus confirm that non–host-reactive donor CD8 cells can play an important role in the regulation of marrow rejection and identify the Tc2 subset of CD8 cells as a population particularly enriched in its ability to facilitate alloengraftment.

The marked ability of the Tc2 population to facilitate alloengraftment indicates that the process of graft rejection is quite susceptible to regulation by non–host-reactive donor T cells. Previous marrow rejection studies using unmanipulated donor CD8⁺ T cells indicated that non–host-reactive CD8 cells were less potent than donor CD8 cells capable of mediating an alloaggressive response against the host.[14] In this study, we have demonstrated that the non–host-reactive mechanism for preventing graft rejection can be augmented by using in vitro-generated donor CD8 cells enriched for cytotoxic function and type II

cytokine secretion. Because host-reactive T-cell responses result in GVHD, use of Tc2-type donor populations to abrogate rejection via a non–host-reactive pathway represents a new strategy for improving the balance between alloengraftment and GVHD. In previous studies, we have demonstrated that host-reactive CD8⁺ T cells of Tc2 phenotype can mediate a GVL effect with reduced GVHD relative to unmanipulated donor T cells[8]; given these results, we would predict that host-reactive Tc2 cells might also represent a strategy for preventing rejection with reduced GVHD.

Previous studies have indicated that cytolytic function and an ability to clonally delete allospecific precursor CTL might be two parameters predictive for an ability to prevent graft rejection by a non–host-reactive, veto-type mechanism.[12] Given that the Tc1- and Tc2-type donor CD8⁺ T cells possessed similar cytolytic function and ability to clonally delete allospecific precursor CTL, we reasoned that these CD8 subsets would prevent marrow rejection in this F1 into-parent model with similar efficacy. However, because the Tc2 subset was clearly superior in preventing marrow graft rejection, we believe that other functional characteristics must contribute to the in vivo effectiveness of this population.

The mechanism for the enhanced ability of the Tc2 subset to abrogate rejection relative to the Tc1 population is currently not known, but likely involves a noncytolytic characteristic of the Tc2-type cells. One possibility is that the Tc2 subset has a longer in vivo half-life or a favorable in vivo homing pattern. A second possibility is that the dichotomous cytokine secretion pattern of the Tc1 and Tc2 subsets might exert differential effects on the graft rejection process in vivo; eg, a murine tumor model has demonstrated that the type II cytokine IL-10 can block an allogeneic rejection response.[26] However, we do not favor this explanation in our experiments, because our previous pilot studies using CD4⁺ Th2 cells, which secreted the type II cytokines but were noncytolytic, did not abrogate marrow rejection in an F1 into-parent model.

It is also possible that the Tc2 cells might be more effective than the Tc1 population at preventing marrow rejection mediated by host CD4⁺ T cells. The graft rejection model used in these studies involves disparities at both MHC class I and class II alloantigens; as such, radioresistant host CD4⁺ and CD8⁺ T cells may both have contributed to the marrow rejection process. In contrast to human CD8⁺ T cells, which can express HLA class II antigens, murine CD8⁺ T cells are not known to express class II antigens; the Tc1 and Tc2 populations used in these studies were negative for MHC class II expression by routine FCM analysis. Because the classical veto mechanism is driven by host recognition of donor antigens, if Tc2 cells are capable of preventing CD4-mediated rejection, an alternative (non-veto) mechanism would presumably be operative.

Clinical translation of the non–host-reactive Tc2 strategy would require the development of methodologies for the generation and characterization of human Tc2 cells that are rendered nonreactive to host alloantigens. Application of this strategy in the setting of transplantation for nonmalignant diseases would likely result in a favorable balance between engraftment and GVHD. However, because the GVL effect may require an alloaggressive T-cell reaction, the use of host-

reactive Tc2 cells for the purpose of abrogating rejection might be advantageous in the setting of leukemic hosts.

In conclusion, we have identified the Tc2 subset of donor CD8⁺ T cells as a population particularly enriched in its ability to abrogate marrow graft rejection. Our observation that the Tc2 population abrogates rejection without inducing an alloaggressive response helps define the biology of T-cell regulation of marrow graft rejection and offers a new strategy for achieving alloengraftment with reduced GVHD. Combined with our previous findings that host-reactive Tc2 cells can mediate a GVL effect with reduced GVHD, the current results suggest that donor cells of Tc2 phenotype may be the optimal CD8⁺ T-cell subset for use in the setting of allogeneic bone marrow transplantation. Marrow supplemented with both host-reactive and non-host-reactive Tc2 cells might optimally mediate antileukemia effects and prevent marrow rejection. As such, both cytokine phenotype (type I *v* type II) and specificity (host-reactive *v* non-host-reactive) of donor CD8⁺ T cells are important considerations in attempts to broaden the future clinical applicability of allogeneic bone marrow transplantation.

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